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(54) Title: **AROMATIC METHYLTRANSFERASES AND USES THEREOF**

(57) Abstract: The present invention relates to genes associated with the tocopherol biosynthesis pathway. More particularly, the present invention provides and includes nucleic acid molecules, proteins, and antibodies associated with genes that encode polypeptides that have methyltransferase activity. The present invention also provides methods for utilizing such agents, for example in gene isolation, gene analysis and the production of transgenic plants. Moreover, the present invention includes transgenic plants modified to express the aforementioned polypeptides. In addition, the present invention includes methods for the production of products from the tocopherol biosynthesis pathway.

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AROMATIC METHYLTRANSFERASES AND USES THEREOF

5 The present invention is in the field of plant genetics and biochemistry. More specifically, the invention relates to genes associated with the tocopherol biosynthesis pathway, namely those encoding methyltransferase activity, and uses of such genes.

Tocopherols are an important component of mammalian diets. Epidemiological evidence indicates that tocopherol supplementation can result in decreased risk for
10 cardiovascular disease and cancer, can aid in immune function, and is associated with prevention or retardation of a number of degenerative disease processes in humans (Traber and Sies, *Annu. Rev. Nutr.* 16:321-347 (1996)). Tocopherol functions, in part, by stabilizing the lipid bilayer of biological membranes (Skrypin and Kagan, *Biochim. Biophys. Acta* 815:209 (1995); Kagan, *N.Y. Acad. Sci.* p 121, (1989); Gomez-Fernandez *et al.*, *Ann. N.Y. Acad. Sci.* p 109 (1989)), reducing polyunsaturated fatty acid (PUFA) free
15 radicals generated by lipid oxidation (Fukuzawa *et al.*, *Lipids* 17:511-513 (1982)), and scavenging oxygen free radicals, lipid peroxy radicals and singlet oxygen species (Diplock *et al. Ann. N Y Acad. Sci.* 570:72 (1989); Fryer, *Plant Cell Environ.* 15(4):381-392 (1992)).

The compound α -tocopherol, which is often referred to as vitamin E, belongs to a
20 class of lipid-soluble antioxidants that includes α , β , γ , and δ -tocopherols and α , β , γ , and δ -tocotrienols. Although α , β , γ , and δ -tocopherols and α , β , γ , and δ -tocotrienols are sometimes referred to collectively as "vitamin E", vitamin E is more appropriately defined chemically as α -tocopherol. Vitamin E, or α -tocopherol, is significant for human health, in part because it is readily absorbed and retained by the body, and therefore has a higher
25 degree of bioactivity than other tocopherol species (Traber and Sies, *Annu. Rev. Nutr.* 16:321-347 (1996)). However, other tocopherols such as β , γ , and δ -tocopherols also have significant health and nutritional benefits.

Tocopherols are primarily synthesized only by plants and certain other photosynthetic organisms, including cyanobacteria. As a result, mammalian dietary

tocopherols are obtained almost exclusively from these sources. Plant tissues vary considerably in total tocopherol content and tocopherol composition, with α -tocopherol the predominant tocopherol species found in green, photosynthetic plant tissues. Leaf tissue can contain from 10-50 μ g of total tocopherols per gram fresh weight, but most of the world's major staple crops (e.g., rice, corn, wheat, potato) produce low to extremely low levels of total tocopherols, of which only a small percentage is α -tocopherol (Hess, Vitamin E, α -tocopherol, *Antioxidants in Higher Plants*, R. Alscher and J. Hess, Eds., CRC Press, Boca Raton. pp. 111-134 (1993)). Oil seed crops generally contain much higher levels of total tocopherols, but α -tocopherol is present only as a minor component in most oilseeds (Taylor and Barnes, *Chem Ind.*, Oct:722-726 (1981)).

The recommended daily dietary intake of 15-30 mg of vitamin E is quite difficult to achieve from the average American diet. For example, it would take over 750 grams of spinach leaves, in which α -tocopherol comprises 60% of total tocopherols, or 200-400 grams of soybean oil to satisfy this recommended daily vitamin E intake. While it is possible to augment the diet with supplements, most of these supplements contain primarily synthetic vitamin E, having eight stereoisomers, whereas natural vitamin E is predominantly composed of only a single isomer. Furthermore, supplements tend to be relatively expensive, and the general population is disinclined to take vitamin supplements on a regular basis. Therefore, there is a need in the art for compositions and methods that either increase the total tocopherol production or increase the relative percentage of α -tocopherol produced by plants.

In addition to the health benefits of tocopherols, increased α -tocopherol levels in crops have been associated with enhanced stability and extended shelf life of plant products (Peterson, *Cereal-Chem.* 72(1):21-24 (1995); Ball, *Fat-soluble vitamin assays in food analysis. A comprehensive review*, London, Elsevier Science Publishers Ltd. (1988)). Further, tocopherol supplementation of swine, beef, and poultry feeds has been shown to significantly increase meat quality and extend the shelf life of post-processed meat products by retarding post-processing lipid oxidation, which contributes to the undesirable flavor components (Sante and Lacourt, *J. Sci. Food Agric.* 65(4):503-507 (1994); Buckley et al., *J. of Animal Science* 73:3122-3130 (1995)).

TOCOPHEROL BIOSYNTHESIS

The plastids of higher plants exhibit interconnected biochemical pathways leading to secondary metabolites including tocopherols. The tocopherol biosynthetic pathway in higher plants involves condensation of homogentisic acid and phytylpyrophosphate to form 2-methylphytylplastoquinol (Fiedler *et al.*, *Planta* 155:511-515 (1982); Soll *et al.*, *Arch. Biochem. Biophys.* 204:544-550 (1980); Marshall *et al.*, *Phytochem.* 24:1705-1711 (1985)). This plant tocopherol pathway can be divided into four parts: 1) synthesis of homogentisic acid (HGA), which contributes to the aromatic ring of tocopherol; 2) synthesis of phytylpyrophosphate, which contributes to the side chain of tocopherol; 3) joining of HGA and phytylpyrophosphate via a prenyltransferase followed by a subsequent cyclization; 4) and S-adenosyl methionine dependent methylation of an aromatic ring, which affects the relative abundance of each of the tocopherol species. See Figure 1.

Various genes and their encoded proteins that are involved in tocopherol biosynthesis are listed in the table below.

Gene ID or Enzyme Abbreviation	Enzyme name
<i>tyrA</i>	Bifunctional Prephenate dehydrogenase
<i>HPT</i>	Homogentisate phytyl transferase
<i>DXS</i>	1-Deoxyxylulose-5- phosphate synthase
<i>DXR</i>	1-Deoxyxylulose-5- phosphate reductoisomerase
<i>GGPPS</i>	Geranylgeranyl pyrophosphate synthase
<i>HPPD</i>	p-Hydroxyphenylpyruvate dioxygenase
<i>AANT1</i>	Adenylate transporter
<i>IDI</i>	Isopentenyl diphosphate isomerase
<i>MT1</i>	Methyl transferase 1
<i>tMT2</i>	Tocopherol methyl transferase 2
<i>GGH</i>	Geranylgeranyl diphosphate reductase
<i>slr1737</i>	Tocopherol cyclase
<i>GMT</i>	Gamma Methyl Transferase

As used herein, homogentisate phytyl transferase (HPT), phytylprenyl transferase (PPT), slr1736, and ATPT2, each refer to proteins or genes encoding proteins that have the same enzymatic activity.

SYNTHESIS OF HOMOGENTISIC ACID

Homogentisic acid is the common precursor to both tocopherols and plastoquinones. In at least some bacteria the synthesis of homogentisic acid is reported to occur via the conversion of chorismate to prephenate and then to p-hydroxyphenylpyruvate via a bifunctional prephenate dehydrogenase. Examples of bifunctional bacterial prephenate dehydrogenase enzymes include the proteins encoded by the *tyrA* genes of *Erwinia herbicola* and *Escherichia coli*. The *tyrA* gene product catalyzes the production of prephenate from chorismate, as well as the subsequent dehydrogenation of prephenate to form p-hydroxyphenylpyruvate (p-HPP), the immediate precursor to homogentisic acid. p-HPP is then converted to homogentisic acid by hydroxyphenylpyruvate dioxygenase (HPPD). In contrast, plants are believed to lack prephenate dehydrogenase activity, and it is generally believed that the synthesis of homogentisic acid from chorismate occurs via the synthesis and conversion of the intermediate arogenate. Since pathways involved in homogentisic acid synthesis are also responsible for tyrosine formation, any alterations in these pathways can also result in the alteration in tyrosine synthesis and the synthesis of other aromatic amino acids.

SYNTHESIS OF PHYTYLPYROPHOSPHATE

Tocopherols are a member of the class of compounds referred to as the isoprenoids. Other isoprenoids include carotenoids, gibberellins, terpenes, chlorophyll and abscisic acid. A central intermediate in the production of isoprenoids is isopentenyl diphosphate (IPP). Cytoplasmic and plastid-based pathways to generate IPP have been reported. The cytoplasmic based pathway involves the enzymes acetoacetyl CoA thiolase, HMGCoA synthase, HMGCoA reductase, mevalonate kinase, phosphomevalonate kinase, and mevalonate pyrophosphate decarboxylase.

Recently, evidence for the existence of an alternative, plastid based, isoprenoid biosynthetic pathway emerged from studies in the research groups of Rohmer and Arigoni (Eisenreich *et al.*, *Chem. Bio.*, 5:R221-R233 (1998); Rohmer, *Prog. Drug. Res.*, 50:135-154 (1998); Rohmer, *Comprehensive Natural Products Chemistry*, Vol. 2, pp. 45-68, Barton and Nakanishi (eds.), Pergamon Press, Oxford, England (1999)), who found that the isotope labeling patterns observed in studies on certain eubacterial and plant terpenoids could not be explained in terms of the mevalonate pathway. Arigoni and coworkers

subsequently showed that 1-deoxyxylulose, or a derivative thereof, serves as an intermediate of the novel pathway, now referred to as the MEP pathway (Rohmer *et al.*, *Biochem. J.*, 295:517-524 (1993); Schwarz, Ph.D. thesis, Eidgenössische Technische Hochschule, Zurich, Switzerland (1994)). Recent studies showed the formation of 1-
5 deoxyxylulose 5-phosphate (Broers, Ph.D. thesis (Eidgenössische Technische Hochschule, Zurich, Switzerland) (1994)) from one molecule each of glyceraldehyde 3-phosphate (Rohmer, *Comprehensive Natural Products Chemistry*, Vol. 2, pp. 45-68, Barton and Nakanishi, eds., Pergamon Press, Oxford, England (1999)) and pyruvate (Eisenreich *et al.*, *Chem. Biol.*, 5:R223-R233 (1998); Schwarz *supra*; Rohmer *et al.*, *J. Am. Chem. Soc.*,
10 118:2564-2566 (1996); and Sprenger *et al.*, *Proc. Natl. Acad. Sci. USA*, 94:12857-12862 (1997)) by an enzyme encoded by the *dxs* gene (Lois *et al.*, *Proc. Natl. Acad. Sci. USA*, 95:2105-2110 (1997); and Lange *et al.*, *Proc. Natl. Acad. Sci. USA*, 95:2100-2104 (1998)). 1-Deoxyxylulose 5-phosphate can be further converted into 2-C-methylerythritol 4-phosphate (Arigoni *et al.*, *Proc. Natl. Acad. Sci. USA*, 94:10600-10605 (1997)) by a
15 reductoisomerase encoded by the *dxr* gene (Bouvier *et al.*, *Plant Physiol*, 117:1421-1431 (1998); and Rohdich *et al.*, *Proc. Natl. Acad. Sci. USA*, 96:11758-11763 (1999)).

Reported genes in the MEP pathway also include *ygbP*, which catalyzes the conversion of 2-C-methylerythritol 4-phosphate into its respective cytidyl pyrophosphate derivative and *ygbB*, which catalyzes the conversion of 4-phosphocytidyl-2C-methyl-D-
20 erythritol into 2C-methyl-D-erythritol, 3, 4-cyclophosphate. These genes are tightly linked on the *E. coli* genome (Herz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 97(6):2485-2490 (2000)).

Once IPP is formed by the MEP pathway, it is converted to GGDP by GGDP synthase, and then to phytylpyrophosphate, which is the central constituent of the tocopherol side chain.

25 COMBINATION AND CYCLIZATION

Homogentisic acid is combined with either phytyl-pyrophosphate or solanyl-pyrophosphate by phytyl/prenyl transferase forming 2-methylphytyl plastoquinol or 2-methylsolanyl plastoquinol, respectively. 2-methylsolanyl plastoquinol is a precursor to the biosynthesis of plastoquinones, while 2-methylphytyl plastoquinol is ultimately
30 converted to tocopherol.

METHYLATION OF THE AROMATIC RING

The major structural difference between each of the tocopherol subtypes is the position of the methyl groups around the phenyl ring. Both 2-methylphytyl plastoquinol and 2-methylsolanyl plastoquinol serve as substrates for the plant enzyme 2-methylphytylplastoquinol/2-methylsolanylplastoquinol methyltransferase (Tocopherol Methyl Transferase 2; Methyl Transferase 2; MT2; tMT2), which is capable of methylating a tocopherol precursor. Subsequent methylation at the 5 position of γ -tocopherol by γ -tocopherol methyl-transferase (GMT) generates the biologically active α -tocopherol.

A possible alternate pathway for the generation of α -tocopherol involves the generation of δ -tocopherol via the cyclization of 2-methylphytylplastoquinol by tocopherol cyclase. δ -tocopherol is then converted to β -tocopherol via the methylation of the 5 position by GMT. δ -tocopherol can be converted to α -tocopherol via methylation of the 3 position by tMT2, followed by methylation of the 5 position by GMT. In a possible alternative pathway, β -tocopherol is directly converted to α -tocopherol by tMT2 via the methylation of the 3 position (see, for example, *Biochemical Society Transactions*, 11:504-510 (1983); *Introduction to Plant Biochemistry*, 2nd edition, chapter 11 (1983); *Vitamin Hormone*, 29:153-200 (1971); *Biochemical Journal*, 109:577 (1968); and, *Biochemical and Biophysical Research Communication*, 28(3):295 (1967)). Since all potential mechanisms for the generation of α -tocopherol involve catalysis by tMT2, plants that are deficient in this activity accumulate δ -tocopherol and β -tocopherol. Plants which have increased tMT2 activity tend to accumulate γ -tocopherol and α -tocopherol. Since there is no GMT activity in the seeds of many plants, these plants tend to accumulate γ -tocopherol.

There is a need in the art for nucleic acid molecules encoding enzymes involved in tocopherol biosynthesis, as well as related enzymes and antibodies for the enhancement or alteration of tocopherol production in plants. There is a further need for transgenic organisms expressing those nucleic acid molecules involved in tocopherol biosynthesis, which are capable of nutritionally enhancing food and feed sources.

BRIEF SUMMARY OF THE INVENTION

The present invention includes and provides a substantially purified nucleic acid molecule encoding a tMT2 enzyme.

The present invention includes and provides a substantially purified nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1 and 2.

5 The present invention includes and provides a substantially purified nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 3 through 7.

The present invention includes and provides a substantially purified nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 8 through 14.

10 The present invention includes and provides a substantially purified nucleic acid molecule encoding a plant polypeptide molecule having 2-Methylphytylplastoquinol methyltransferase activity.

The present invention includes and provides a substantially purified plant polypeptide molecule having 2-Methylphytylplastoquinol methyltransferase activity.

15 The present invention includes and provides a substantially purified mutant polypeptide molecule having an altered 2-Methylphytylplastoquinol methyltransferase activity relative to a non-mutant polypeptide.

The present invention includes and provides a substantially purified polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16 and 28.

The present invention includes and provides a substantially purified polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 17 through 21 and 29 through 32.

25 The present invention includes and provides a substantially purified polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 22 through 27 and 33 through 38.

The present invention includes and provides an antibody capable of specifically binding a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16 through 38.

The present invention includes and provides a transformed plant comprising an introduced nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof.

5 The present invention includes and provides a transformed plant comprising an introduced nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, and 33 through 38.

10 The present invention includes and provides a transformed plant comprising a nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 17 through 21, and 29 through 32.

15 The present invention includes and provides a transformed plant comprising an introduced first nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof, and an introduced second nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA*, *slr1736*, *HPT*, *GMT*, *tocopherol cyclase*, *dxs*, *dxr*, *GGPPS*, *HPPD*, *AANT1*, *slr1737*, *IDI*, *GGH*, and complements thereof, a plant ortholog thereof, and an antisense construct for homogentisic acid dioxygenase.

20 The present invention includes and provides a transformed plant comprising an introduced first nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, 33 through 38, and an introduced second nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA*, *slr1736*, *HPT*, *GMT*, *tocopherol cyclase*, *dxs*, *dxr*, *GGPPS*, *HPPD*, *AANT1*, *slr1737*, *IDI*, *GGH*, and complements thereof, a plant
25 ortholog thereof, and an antisense construct for homogentisic acid dioxygenase.

The present invention includes and provides a transformed plant comprising an introduced first nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof and an introduced second nucleic acid molecule comprising a sequence selected from the group
30 consisting of SEQ ID NOs: 39 through 54, and complements thereof.

The present invention includes and provides a transformed plant comprising an introduced first nucleic acid molecule that encodes a polypeptide molecule comprising an

amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, 33 through 38, and an introduced second nucleic acid molecule having a sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof.

5 The present invention includes and provides a method for reducing expression of the tMT2 gene in a plant comprising: (A) transforming a plant with a nucleic acid molecule, said nucleic acid molecule having an introduced promoter region which functions in plant cells to cause the production of a mRNA molecule, wherein said introduced promoter region is linked to a transcribed nucleic acid molecule having a
10 transcribed strand and a non-transcribed strand, wherein said transcribed strand is complementary to a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 through 15, and wherein said transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the plant cells to cause termination of transcription and addition of polyadenylated ribonucleotides to
15 a 3' end of the mRNA sequence; and (B) growing said transformed plant.

 The present invention includes and provides a transformed plant comprising a nucleic acid molecule comprising an introduced promoter region which functions in plant cells to cause the production of an mRNA molecule, wherein said introduced promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a
20 non-transcribed strand, wherein said transcribed strand is complementary to a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 through 15, and wherein said transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the plant cells to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA
25 sequence.

 The present invention includes and provides a method of producing a plant having a seed with an increased γ -tocopherol level comprising: (A) transforming said plant with an introduced nucleic acid molecule, wherein said nucleic acid molecule comprises a sequence encoding a polypeptide molecule comprising an amino acid sequence selected from the
30 group consisting of SEQ ID NOs: 16, 22 through 28, and 33 through 38; and (B) growing said transformed plant.

The present invention includes and provides a method of producing a plant having a seed with an increased γ -tocopherol level comprising: (A) transforming said plant with an introduced first nucleic acid molecule, wherein said first nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and an introduced second nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA*, *slr1736*, *HPT*, *GMT*, *tocopherol cyclase*, *dxs*, *dxr*, *GGPPS*, *HPPD*, *AANT1*, *slr1737*, *IDI*, *GGH*, and complements thereof, a plant ortholog thereof, and an antisense construct for homogentisic acid dioxygenase; and (B) growing said transformed plant.

The present invention includes and provides a method of producing a plant having a seed with an increased γ -tocopherol level comprising: (A) transforming said plant with an introduced first nucleic acid molecule, wherein said first nucleic acid molecule comprises a sequence encoding a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, 33 through 38, and an introduced second nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA*, *slr1736*, *HPT*, *GMT*, *tocopherol cyclase*, *dxs*, *dxr*, *GGPPS*, *HPPD*, *AANT1*, *slr1737*, *IDI*, *GGH*, and complements thereof, a plant ortholog thereof, and an antisense construct for homogentisic acid dioxygenase; and (B) growing said transformed plant.

The present invention includes and provides a method of producing a plant having a seed with an increased α -tocopherol level comprising: (A) transforming said plant with an introduced first nucleic acid molecule, wherein said first nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and an introduced second nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof; and (B) growing said transformed plant.

The present invention includes and provides a method of producing a plant having a seed with an increased α -tocopherol level comprising: (A) transforming said plant with an introduced first nucleic acid molecule, wherein said first nucleic acid molecule comprises a sequence encoding a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, 33 through 38, and an introduced second nucleic acid molecule comprising a sequence selected from the group

consisting of SEQ ID NOs: 39 through 54, and complements thereof; and (B) growing said transformed plant.

The present invention includes and provides a seed derived from a transformed plant comprising an introduced nucleic acid molecule comprising a nucleic acid sequence
5 selected from the group consisting of SEQ ID NOs: 1, 2, and 8 through 15.

The present invention includes and provides a seed derived from a transformed plant comprising an introduced nucleic acid molecule comprising an introduced first nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and an introduced second nucleic acid encoding an enzyme selected from the group
10 consisting of *tyrA*, *slr1736*, *HPT*, *GMT*, *tocopherol cyclase*, *dxs*, *dxr*, *GGPPS*, *HPPD*, *AANT1*, *slr1737*, *IDI*, *GGH*, and complements thereof, a plant ortholog thereof, and an antisense construct for homogentisic acid dioxygenase.

The present invention includes and provides a seed derived from a transformed plant comprising an introduced first nucleic acid molecule comprising a nucleic acid
15 sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and an introduced second nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 39 through 54.

The present invention includes and provides a transformed plant comprising an introduced first nucleic acid molecule comprising a sequence selected from the group
20 consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof, and an introduced second nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof, and an introduced third nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA*, *slr1736*, *HPT*, *GMT*, *tocopherol cyclase*, *dxs*, *dxr*, *GGPPS*, *HPPD*, *AANT1*, *slr1737*, *IDI*, *GGH*, and
25 complements thereof, a plant ortholog thereof, and an antisense construct for homogentisic acid dioxygenase.

The present invention includes and provides a transformed plant comprising an introduced first nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through
30 28, 33 through 38, an introduced second nucleic acid molecule having a sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof, and an introduced third nucleic acid molecule encoding an enzyme selected from the group

consisting of *tyrA*, *slr1736*, *HPT*, *GMT*, *tocopherol cyclase*, *dxs*, *dxr*, *GGPPS*, *HPPD*, *AANT1*, *slr1737*, *IDI*, *GGH*, and complements thereof.

The present invention includes and provides a transformed plant comprising an introduced first nucleic acid molecule encoding a tMT2 enzyme, and a second nucleic acid molecule encoding a GMT enzyme.

The present invention includes and provides a method of producing a plant having seed with an increased α -tocopherol level comprising: (A) transforming said plant with a nucleic acid molecule encoding a tMT2 enzyme and a nucleic acid molecule encoding a GMT enzyme; and (B) growing said plant.

BRIEF DESCRIPTION OF THE NUCLEIC AND AMINO ACID SEQUENCES

SEQ ID NO: 1 sets forth a nucleic acid sequence of a DNA molecule that encodes a wild type *Arabidopsis thaliana*, Columbia ecotype, tMT2 enzyme.

SEQ ID NO: 2 sets forth a nucleic acid sequence of a DNA molecule that encodes a wild type *Arabidopsis thaliana*, Landsberg ecotype, tMT2 enzyme.

SEQ ID NO: 3 sets forth a nucleic acid sequence of a DNA molecule that encodes an hdt2 mutant of the *Arabidopsis thaliana*, Landsberg ecotype, tMT2 enzyme.

SEQ ID NO: 4 sets forth a nucleic acid sequence of a DNA molecule that encodes an hdt6 mutant of the *Arabidopsis thaliana*, Columbia ecotype, tMT2 enzyme.

SEQ ID NO: 5 sets forth a nucleic acid sequence of a DNA molecule that encodes an hdt9 mutant of the *Arabidopsis thaliana*, Columbia ecotype, tMT2 enzyme.

SEQ ID NO: 6 sets forth a nucleic acid sequence of a DNA molecule that encodes an hdt10 mutant of the *Arabidopsis thaliana*, Landsberg ecotype, tMT2 enzyme.

SEQ ID NO: 7 sets forth a nucleic acid sequence of a DNA molecule that encodes an hdt16 mutant of the *Arabidopsis thaliana*, Columbia ecotype, tMT2 enzyme.

SEQ ID NO: 8 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Zea mays* tMT2 enzyme.

SEQ ID NO: 9 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Gossypium hirsutum* tMT2 enzyme.

SEQ ID NO: 10 sets forth a nucleic acid sequence of a DNA molecule that encodes

an *Allium porrum* tMT2 enzyme.

SEQ ID NO: 11 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Glycine max* tMT2 enzyme.

5 SEQ ID NO: 12 sets forth a nucleic acid sequence of a DNA molecule that encodes an *Oryza sativa* tMT2 enzyme.

SEQ ID NO: 13 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Brassica napus* tMT2 enzyme.

SEQ ID NO: 14 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Brassica napus* tMT2 enzyme different in sequence from SEQ ID NO: 13.

10 SEQ ID NO: 15 sets forth a nucleic acid coding sequence of a wild type *Arabidopsis thaliana* tMT2 enzyme.

SEQ ID NO: 16 sets forth an amino acid sequence of a wild type *Arabidopsis thaliana*, Columbia and Landsberg ecotype, tMT2 enzyme.

15 SEQ ID NO: 17 sets forth an amino acid sequence of an hdt2 mutant of the *Arabidopsis thaliana*, Landsberg ecotype, tMT2 enzyme.

SEQ ID NO: 18 sets forth an amino acid sequence of an hdt6 mutant of the *Arabidopsis thaliana*, Columbia ecotype, tMT2 enzyme.

SEQ ID NO: 19 sets forth an amino acid sequence of an hdt9 mutant of the *Arabidopsis thaliana*, Columbia ecotype, tMT2 enzyme.

20 SEQ ID NO: 20 sets forth an amino acid sequence of an hdt10 mutant of the *Arabidopsis thaliana*, Landsberg ecotype, tMT2 enzyme.

SEQ ID NO: 21 sets forth an amino acid sequence of an hdt16 mutant of the *Arabidopsis thaliana*, Columbia ecotype, tMT2 enzyme.

SEQ ID NO: 22 sets forth an amino acid sequence of a *Zea mays* tMT2 enzyme.

25 SEQ ID NO: 23 sets forth an amino acid sequence of a *Gossypium hirsutum* tMT2 enzyme.

SEQ ID NO: 24 sets forth an amino acid sequence of an *Allium porrum* tMT2 enzyme.

SEQ ID NO: 25 sets forth an amino acid sequence of a *Glycine max* tMT2 enzyme.

SEQ ID NO: 26 sets forth an amino acid sequence of an *Oryza sativa* tMT2 enzyme.

SEQ ID NO: 27 sets forth an amino acid sequence of a *Brassica napus* tMT2 enzyme.

5 SEQ ID NO: 28 sets forth an amino acid sequence of a mature wild type *Arabidopsis thaliana*, Columbia ecotype, tMT2 enzyme.

SEQ ID NO: 29 sets forth an amino acid sequence of a mature hdt2 mutant of the *Arabidopsis thaliana*, Landsberg ecotype, tMT2 enzyme.

10 SEQ ID NO: 30 sets forth an amino acid sequence of a mature hdt6 mutant of the *Arabidopsis thaliana*, Columbia ecotype, tMT2 enzyme.

SEQ ID NO: 31 sets forth an amino acid sequence of a mature hdt10 mutant of the *Arabidopsis thaliana*, Landsberg ecotype, tMT2 enzyme.

SEQ ID NO: 32 sets forth an amino acid sequence of a mature hdt16 mutant of the *Arabidopsis thaliana*, Columbia ecotype, tMT2 enzyme.

15 SEQ ID NO: 33 sets forth an amino acid sequence of a mature *Brassica napus* tMT2 enzyme.

SEQ ID NO: 34 sets forth an amino acid sequence of a mature *Oryza sativa* tMT2 enzyme.

20 SEQ ID NO: 35 sets forth an amino acid sequence of a mature *Zea mays* tMT2 enzyme.

SEQ ID NO: 36 sets forth an amino acid sequence of a mature *Glycine max* tMT2 enzyme.

SEQ ID NO: 37 sets forth an amino acid sequence of a mature *Allium porrum* tMT2 enzyme.

25 SEQ ID NO: 38 sets forth an amino acid sequence of a mature *Gossypium hirsutum* tMT2 enzyme.

SEQ ID NO: 39 sets forth a nucleic acid sequence of a DNA molecule that encodes an *Arabidopsis thaliana* γ -tocopherol methyltransferase.

SEQ ID NO: 40 sets forth a nucleic acid sequence of a DNA molecule that encodes an *Arabidopsis thaliana*, Columbia ecotype, γ -tocopherol methyltransferase.

SEQ ID NO: 41 sets forth a nucleic acid sequence of a DNA molecule that encodes an *Oryza sativa* γ -tocopherol methyltransferase.

5 SEQ ID NO: 42 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Zea mays* γ -tocopherol methyltransferase.

SEQ ID NO: 43 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Gossypium hirsutum* γ -tocopherol methyltransferase.

10 SEQ ID NO: 44 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Cuphea pulcherrima* γ -tocopherol methyltransferase.

SEQ ID NO: 45 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Brassica napus* S8 γ -tocopherol methyltransferase.

SEQ ID NO: 46 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Brassica napus* P4 γ -tocopherol methyltransferase.

15 SEQ ID NO: 47 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Lycopersicon esculentum* γ -tocopherol methyltransferase.

SEQ ID NO: 48 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Glycine max* γ -tocopherol methyltransferase 1.

20 SEQ ID NO: 49 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Glycine max* γ -tocopherol methyltransferase 2.

SEQ ID NO: 50 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Glycine max* γ -tocopherol methyltransferase 3.

SEQ ID NO: 51 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Tagetes erecta* γ -tocopherol methyltransferase.

25 SEQ ID NO: 52 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Sorghum bicolor* γ -tocopherol methyltransferase

SEQ ID NO: 53 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Nostoc punctiforme* γ -tocopherol methyltransferase.

SEQ ID NO: 54 sets forth a nucleic acid sequence of a DNA molecule that encodes

an *Anabaena* γ -tocopherol methyltransferase.

SEQ ID NOs: 55 and 56 set forth nucleic acid sequences of the MAA21_40_1 primer pair.

5 SEQ ID NOs: 57 and 58 set forth nucleic acid sequences of the MAA21_40_2 primer pair.

SEQ ID NOs: 59 and 60 set forth nucleic acid sequences of the MAA21_40_3 primer pair.

SEQ ID NOs: 61 and 62 set forth nucleic acid sequences of the MAA21_40_4 primer pair.

10 SEQ ID NOs: 63 and 64 set forth nucleic acid sequences of the MAA21_40_5 primer pair.

SEQ ID NOs: 65 and 66 set forth nucleic acid sequences of the MAA21_40_6 primer pair.

15 SEQ ID NOs: 67 and 68 set forth nucleic acid sequences of the MAA21_40_7 primer pair.

SEQ ID NOs: 69 and 70 set forth nucleic acid sequences of the MAA21_40_8 primer pair.

SEQ ID NOs: 71 and 72 set forth nucleic acid sequences of the MAA21_40_9 primer pair.

20 SEQ ID NOs: 73 and 74 set forth nucleic acid sequences of the MAA21_40_10 primer pair.

SEQ ID NOs: 75 and 76 set forth nucleic acid sequences of the MAA21_40_11 primer pair.

25 SEQ ID NOs: 77 and 78 set forth nucleic acid sequences of primers for use in amplifying a gene encoding a mature *Brassica napus* tMT2 enzyme.

SEQ ID NOs: 79 and 80 set forth nucleic acid sequences of primers for use in amplifying a gene encoding a mature *Oryza sativa* tMT2 enzyme.

SEQ ID NOs: 81 and 82 set forth nucleic acid sequences of primers for use in amplifying a gene encoding a mature *Zea mays* tMT2 enzyme.

SEQ ID NOs: 83 and 84 set forth nucleic acid sequences of primers for use in amplifying a gene encoding a mature *Glycine max* tMT2 enzyme.

SEQ ID NOs: 85 and 86 set forth nucleic acid sequences of primers for use in amplifying a gene encoding a mature *Allium porrum* tMT2 enzyme.

5 SEQ ID NOs: 87 and 88 set forth nucleic acid sequences of primers for use in amplifying a gene encoding a mature *Gossypium hirsutum* tMT2 enzyme.

SEQ ID NOs: 89 and 90 set forth nucleic acid sequences of primers #17286 and #17181 for use in amplifying a gene encoding a full length *Arabidopsis thaliana* tMT2 enzyme.

10 SEQ ID NO: 91 sets forth an amino acid sequence of an *Arabidopsis thaliana* γ -tocopherol methyltransferase.

SEQ ID NO: 92 sets forth an amino acid sequence of an *Arabidopsis thaliana*, Columbia ecotype, γ -tocopherol methyltransferase.

15 SEQ ID NO: 93 sets forth an amino acid sequence of an *Oryza sativa* γ -tocopherol methyltransferase.

SEQ ID NO: 94 sets forth an amino acid sequence of a *Zea mays* γ -tocopherol methyltransferase.

SEQ ID NO: 95 sets forth an amino acid sequence of a *Gossypium hirsutum* γ -tocopherol methyltransferase.

20 SEQ ID NO: 96 sets forth an amino acid sequence of a *Cuphea pulcherrima* γ -tocopherol methyltransferase.

SEQ ID NO: 97 sets forth an amino acid sequence of a *Brassica napus* S8 γ -tocopherol methyltransferase.

25 SEQ ID NO: 98 sets forth an amino acid sequence of a *Brassica napus* P4 γ -tocopherol methyltransferase.

SEQ ID NO: 99 sets forth an amino acid sequence of a *Lycopersicon esculentum* γ -tocopherol methyltransferase.

SEQ ID NO: 100 sets forth an amino acid sequence of a *Glycine max* γ -tocopherol methyltransferase 1.

SEQ ID NO: 101 sets forth an amino acid sequence of a *Glycine max* γ -tocopherol methyltransferase 2.

SEQ ID NO: 102 sets forth an amino acid sequence of a *Glycine max* γ -tocopherol methyltransferase 3.

5 SEQ ID NO: 103 sets forth an amino acid sequence of a *Tagetes erecta* γ -tocopherol methyltransferase.

SEQ ID NO: 104 sets forth an amino acid sequence of a *Sorghum bicolor* γ -tocopherol methyltransferase.

10 SEQ ID NO: 105 sets forth an amino acid sequence of a *Lilium asiaticum* γ -tocopherol methyltransferase.

SEQ ID NO: 106 sets forth an amino acid sequence of a *Nostoc punctiforme* γ -tocopherol methyltransferase.

SEQ ID NO: 107 sets forth an amino acid sequence of an *Anabaena* γ -tocopherol methyltransferase.

15 tocopherol methyltransferase.

SEQ ID NO: 108 sets forth an amino acid consensus sequence for the aligned polypeptides shown in Figures 3a and 3b.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a schematic diagram of the tocopherol biosynthetic pathway.

20 Figure 2 represents the results of a TBLASTN homology comparison of the nucleotide sequences of several crop tMT2 genes to the amino acid sequence of a tMT2 gene from *Arabidopsis thaliana* (NCBI General Identifier Number gi7573324).

Figures 3a and 3b represent the Pretty Alignment (Genetics Computer Group, Madison WI) of tMT2 protein sequences from different plant species.

25 Figure 4 represents a graph depicting the methyltransferase activity of recombinantly expressed *Anabaena* MT1 (positive control). Enzyme activity is monitored on crude cell extracts from *E. coli* harboring pMON67174.

Figure 5 represents a graph depicting the methyltransferase activity of recombinantly expressed mature *Arabidopsis* tMT2. Enzyme activity is monitored on crude cell extracts from *E. coli* harboring pMON67191.

5 Figure 6 represents a graph depicting the methyltransferase activity of recombinantly expressed mature *Arabidopsis* tMT2 hdt2 mutant. Enzyme activity is monitored on crude cell extracts from *E. coli* harboring pMON67207.

10 Figure 7 represents a graph depicting the methyltransferase activity of recombinantly expressed *Anabaena* MT1 without 2-methylphytylplastoquinol substrate (negative control). Enzyme activity is monitored on crude cell extracts from *E. coli* harboring pMON67174.

Figure 8 represents a graph depicting the methyltransferase I activity in isolated pea chloroplasts (positive control).

Figure 9 is a plasmid map of pMON67205.

Figure 10 is a plasmid map of pMON67220.

15 Figure 11 is a plasmid map of pMON67226.

Figure 12 is a plasmid map of pMON67225.

Figure 13 is a plasmid map of pMON67227.

Figure 14 is a plasmid map of pMON67224.

Figure 15 is a plasmid map of pMON67223.

20 Figures 16a and 16b depict the levels of expression of δ -tocopherol in various types of *Arabidopsis*.

Figure 17 depicts T3 seed δ -tocopherol (%) from two lines expressing tMT2 under the control of the napin promoter (pMON67205) in the *hdt2* mutant line.

25 Figures 18a-d depict the levels of α , β , γ , and δ -tocopherol in tMT2 pools of 10 seeds.

Figures 19a-d depict the levels of α , β , γ , and δ -tocopherol in tMT2/GMT pools of 10 seeds.

Figure 20 depicts the tocopherol composition of single seeds from one line of soybean (28072) transformed with pMON67226.

Figures 21a-d depict the levels of α , β , γ , and δ -tocopherol in R1 Soy Single Seed from pMON67226.

Figure 22 depicts the tocopherol composition of single seeds from one line of soybean (28906) transformed with pMON67227.

5 Figures 23a-d depict the levels of α , β , γ , and δ -tocopherol in R1 Soy Single Seed from pMON67227.

Figure 24 depicts the results of various 2-methylphytylplastoquinol methyltransferase assays.

DETAILED DESCRIPTION OF THE INVENTION

10 The present invention provides a number of agents, for example, nucleic acid molecules and polypeptides associated with the synthesis of tocopherol, and provides uses of such agents.

AGENTS

15 The agents of the invention will preferably be "biologically active" with respect to either a structural attribute, such as the capacity of a nucleic acid to hybridize to another nucleic acid molecule, or the ability of a protein to be bound by an antibody (or to compete with another molecule for such binding). Alternatively, such an attribute may be catalytic and thus involve the capacity of the agent to mediate a chemical reaction or response. The agents will preferably be "substantially purified." The term "substantially purified," as
20 used herein, refers to a molecule separated from substantially all other molecules normally associated with it in its native environmental conditions. More preferably a substantially purified molecule is the predominant species present in a preparation. A substantially purified molecule may be greater than 60% free, preferably 75% free, more preferably 90% free, and most preferably 95% free from the other molecules (exclusive of solvent) present
25 in the natural mixture. The term "substantially purified" is not intended to encompass molecules present in their native environmental conditions.

The agents of the invention may also be recombinant. As used herein, the term recombinant means any agent (*e.g.*, DNA, peptide *etc.*), that is, or results, however indirectly, from human manipulation of a nucleic acid molecule.

30 It is understood that the agents of the invention may be labeled with reagents that facilitate detection of the agent (*e.g.*, fluorescent labels, Prober *et al.*, *Science* 238:336-340

(1987); Albarella *et al.*, EP 144914; chemical labels, Sheldon *et al.*, U.S. Patent 4,582,789; Albarella *et al.*, U.S. Patent 4,563,417; modified bases, Miyoshi *et al.*, EP 119448).

NUCLEIC ACID MOLECULES

Agents of the invention include nucleic acid molecules. In a preferred aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence, which encodes a tocopherol methyltransferase. As used herein, a tocopherol methyltransferase (tMT2) is any plant protein that is capable of specifically catalyzing the methylation of the 3 position of the phenyl ring of 2-methylphytylplastoquinol, 2-methyl-5-phytylplastoquinol, 2-methyl-3-phytylplastoquinol, δ -tocopherol, or β -tocopherol (see, *Photosyn. Research*, 31:99-111 (1992) and *Phytochemistry* 19:215-218 (1980)). A preferred tMT2 is found in an organism selected from the group consisting of *Arabidopsis*, maize, cotton, leek, soybean, rice, and oilseed rape. An example of a more preferred tMT2 is a polypeptide with the amino acid sequence selected from the group consisting of SEQ ID NOs: 16 through 38. In a more preferred embodiment, the tMT2 is encoded by any of SEQ ID NOs: 1 through 15.

In another preferred aspect of the present invention a nucleic acid molecule of the present invention comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 through 15, and complements thereof and fragments of either. In another preferred aspect of the present invention, a nucleic acid molecule of the present invention comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 and 2, and complements thereof. In another preferred aspect of the present invention the nucleic acid molecule of the invention comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 3 through 7, and complements thereof. In another preferred aspect of the present invention the nucleic acid molecule of the invention comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 8 through 14, and complements thereof. In another preferred aspect of the present invention the nucleic acid molecule of the invention comprises the nucleic acid sequence of SEQ ID NO: 15 and its complement. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 16 through 38, and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding amino acid sequence SEQ ID NO: 16 and fragments thereof.

In another embodiment, the present invention provides nucleic acid molecules comprising a sequence encoding SEQ ID NO: 108, and complements thereof. In another aspect, the present invention provides nucleic acid molecules comprising a sequence encoding residues 83 through 356 of SEQ ID NO: 108, and its complement. In another aspect, the present invention provides nucleic acid molecules comprising a sequence encoding a fragment of residues 83 through 356 of SEQ ID NO: 108, wherein the fragment has a length of at least about 25, 50, 75, 100, 150, 200, or 250 residues, and complements thereof. In yet another aspect, the present invention provides nucleic acid molecules encoding one or more of the following fragments of SEQ ID NO: 108, and complements thereof: 82 through 123, 132 through 146, and 269 through 295.

The present invention includes the use of the above-described sequences and fragments thereof in transgenic plants, other organisms, and for other uses as described below.

In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 17 through 21, and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 22 through 27, and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 28 through 38, and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid of SEQ ID NO: 28 and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 29 through 32, and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 33 through 38, and fragments thereof.

In another preferred aspect of the present invention a nucleic acid molecule comprises nucleotide sequences encoding a plastid transit peptide operably fused to a nucleic acid molecule that encodes a protein or fragment of the present invention.

In another preferred embodiment of the present invention, the nucleic acid molecules of the invention encode mutant tMT2 enzymes. As used herein, a "mutant" enzyme or polypeptide is any enzyme or polypeptide that contains an amino acid that is different from the amino acid in the same position of a wild type enzyme of the same type.

5 Examples of suitable mutants of the invention include, but are not limited to, those found in Example 1 of this application.

It is understood that in a further aspect of nucleic acid sequences of the present invention, the nucleic acids can encode a protein that differs from any of the proteins in that one or more amino acids have been deleted, substituted or added without altering the

10 function. For example, it is understood that codons capable of coding for such conservative amino acid substitutions are known in the art.

In one aspect of the present invention the nucleic acids of the present invention are said to be introduced nucleic acid molecules. A nucleic acid molecule is said to be "introduced" if it is inserted into a cell or organism as a result of human manipulation, no

15 matter how indirect. Examples of introduced nucleic acid molecules include, without limitation, nucleic acids that have been introduced into cells via transformation, transfection, injection, and projection, and those that have been introduced into an organism via conjugation, endocytosis, phagocytosis, etc.

One subset of the nucleic acid molecules of the invention is fragment nucleic acids

20 molecules. Fragment nucleic acid molecules may consist of significant portion(s) of, or indeed most of, the nucleic acid molecules of the invention, such as those specifically disclosed. Alternatively, the fragments may comprise smaller oligonucleotides (having from about 15 to about 400 nucleotide residues and more preferably, about 15 to about 30 nucleotide residues, or about 50 to about 100 nucleotide residues, or about 100 to about

25 200 nucleotide residues, or about 200 to about 400 nucleotide residues, or about 275 to about 350 nucleotide residues).

A fragment of one or more of the nucleic acid molecules of the invention may be a probe and specifically a PCR probe. A PCR probe is a nucleic acid molecule capable of initiating a polymerase activity while in a double-stranded structure with another nucleic

30 acid. Various methods for determining the structure of PCR probes and PCR techniques exist in the art. Computer generated searches using programs such as Primer3 (www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi), STSPipeline (www-

genome.wi.mit.edu/cgi-bin/www-STS_Pipeline), or GeneUp (Pesole *et al.*, *BioTechniques* 25:112-123 (1998)), for example, can be used to identify potential PCR primers.

Nucleic acid molecules or fragments thereof of the present invention are capable of specifically hybridizing to other nucleic acid molecules under certain circumstances.

5 Nucleic acid molecules of the present invention include those that specifically hybridize to nucleic acid molecules having a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 through 15, and complements thereof. Nucleic acid molecules of the present invention also include those that specifically hybridize to nucleic acid molecules encoding an amino acid sequence selected from SEQ ID NOs: 16 through 38, and
10 fragments thereof.

As used herein, two nucleic acid molecules are said to be capable of specifically hybridizing to one another if the two molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure.

A nucleic acid molecule is said to be the "complement" of another nucleic acid
15 molecule if they exhibit complete complementarity. As used herein, molecules are said to exhibit "complete complementarity" when every nucleotide of one of the molecules is complementary to a nucleotide of the other. Two molecules are said to be "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency"
20 conditions. Similarly, the molecules are said to be "complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under conventional "high-stringency" conditions. Conventional stringency conditions are described by Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual, 2nd Ed.*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989), and by Haymes *et al.*,
25 *Nucleic Acid Hybridization, A Practical Approach*, IRL Press, Washington, DC (1985). Departures from complete complementarity are therefore permissible, as long as such departures do not completely preclude the capacity of the molecules to form a double-stranded structure. Thus, in order for a nucleic acid molecule to serve as a primer or probe it need only be sufficiently complementary in sequence to be able to form a stable double-
30 stranded structure under the particular solvent and salt concentrations employed.

Appropriate stringency conditions which promote DNA hybridization are, for example, 6.0 X sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of

2.0 X SSC at 20-25°C, are known to those skilled in the art or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 X SSC at 50°C to a high stringency of about 0.2 X SSC at 65°C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22°C, to high stringency conditions at about 65°C. Both temperature and salt may be varied, or either the temperature or the salt concentration may be held constant while the other variable is changed.

In a preferred embodiment, a nucleic acid of the present invention will specifically hybridize to one or more of the nucleic acid molecules set forth in SEQ ID NOs: 1 through 15, and complements thereof under moderately stringent conditions, for example at about 2.0 X SSC and about 65°C.

In a particularly preferred embodiment, a nucleic acid of the present invention will include those nucleic acid molecules that specifically hybridize to one or more of the nucleic acid molecules set forth in SEQ ID NOs: 1 through 15, and complements thereof under high stringency conditions such as 0.2 X SSC and about 65°C.

In one aspect of the present invention, the nucleic acid molecules of the present invention have one or more of the nucleic acid sequences set forth in SEQ ID NOs: 1 through 15, and complements thereof. In another aspect of the present invention, one or more of the nucleic acid molecules of the present invention share between 100% and 90% sequence identity with one or more of the nucleic acid sequences set forth in SEQ ID NOs: 1 through 15, and complements thereof and fragments of either. In a further aspect of the present invention, one or more of the nucleic acid molecules of the present invention share between 100% and 95% sequence identity with one or more of the nucleic acid sequences set forth in SEQ ID NOs: 1 through 15, complements thereof, and fragments of either. In a more preferred aspect of the present invention, one or more of the nucleic acid molecules of the present invention share between 100% and 98% sequence identity with one or more of the nucleic acid sequences set forth in SEQ ID NOs: 1 through 15, complements thereof and fragments of either. In an even more preferred aspect of the present invention, one or more of the nucleic acid molecules of the present invention share between 100% and 99% sequence identity with one or more of the sequences set forth in SEQ ID NOs: 1 through 15, complements thereof, and fragments of either.

In a preferred embodiment the percent identity calculations are performed using BLASTN or BLASTP (default, parameters, version 2.0.8, Altschul *et al.*, *Nucleic Acids Res.* 25:3389-3402 (1997)).

A nucleic acid molecule of the invention can also encode a homolog polypeptide.

5 As used herein, a homolog polypeptide molecule or fragment thereof is a counterpart protein molecule or fragment thereof in a second species (*e.g.*, corn rubisco small subunit is a homolog of *Arabidopsis* rubisco small subunit). A homolog can also be generated by molecular evolution or DNA shuffling techniques, so that the molecule retains at least one functional or structure characteristic of the original polypeptide (*see*, for example, U.S.
10 Patent 5,811,238).

In another embodiment, the homolog is selected from the group consisting of alfalfa, *Arabidopsis*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, canola, citrus, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato,
15 wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm. More particularly, preferred homologs are selected from canola, corn, *Brassica campestris*, *Brassica napus*, oilseed rape, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, rapeseed, safflower, oil palm, flax, and
20 sunflower. In an even more preferred embodiment, the homolog is selected from the group consisting of canola, rapeseed, corn, *Brassica campestris*, *Brassica napus*, oilseed rape, soybean, sunflower, safflower, oil palms, and peanut. In a particularly preferred embodiment, the homolog is soybean. In a particularly preferred embodiment, the homolog is canola. In a particularly preferred embodiment, the homolog is oilseed rape.

25 In a preferred embodiment, nucleic acid molecules having SEQ ID NOs: 1 through 15, complements thereof, and fragments of either; or more preferably SEQ ID NOs: 1 through 15, and complements thereof, can be utilized to obtain such homologs.

In another further aspect of the present invention, nucleic acid molecules of the present invention can comprise sequences that differ from those encoding a polypeptide or
30 fragment thereof in SEQ ID NOs: 1 through 15 due to the fact that a polypeptide can have one or more conservative amino acid changes, and nucleic acid sequences coding for the

polypeptide can therefore have sequence differences. It is understood that codons capable of coding for such conservative amino acid substitutions are known in the art.

It is well known in the art that one or more amino acids in a native sequence can be substituted with other amino acid(s), the charge and polarity of which are similar to that of the native amino acid, *i.e.*, a conservative amino acid substitution. Conservative substitutes for an amino acid within the native polypeptide sequence can be selected from other members of the class to which the amino acid belongs. Amino acids can be divided into the following four groups: (1) acidic amino acids, (2) basic amino acids, (3) neutral polar amino acids, and (4) neutral, nonpolar amino acids. Representative amino acids within these various groups include, but are not limited to, (1) acidic (negatively charged) amino acids such as aspartic acid and glutamic acid; (2) basic (positively charged) amino acids such as arginine, histidine, and lysine; (3) neutral polar amino acids such as glycine, serine, threonine, cysteine, cystine, tyrosine, asparagine, and glutamine; and (4) neutral nonpolar (hydrophobic) amino acids such as alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine.

Conservative amino acid substitution within the native polypeptide sequence can be made by replacing one amino acid from within one of these groups with another amino acid from within the same group. In a preferred aspect, biologically functional equivalents of the proteins or fragments thereof of the present invention can have ten or fewer conservative amino acid changes, more preferably seven or fewer conservative amino acid changes, and most preferably five or fewer conservative amino acid changes. The encoding nucleotide sequence will thus have corresponding base substitutions, permitting it to encode biologically functional equivalent forms of the polypeptides of the present invention.

It is understood that certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Because it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence and, of course, its underlying DNA coding sequence and, nevertheless, a protein with like properties can still be obtained. It is thus contemplated by the inventors that various changes may be made in the peptide sequences of the proteins or fragments of the present invention, or corresponding DNA sequences that encode said

peptides, without appreciable loss of their biological utility or activity. It is understood that codons capable of coding for such amino acid changes are known in the art.

In making such changes, the hydrophathic index of amino acids may be considered. The importance of the hydrophathic amino acid index in conferring interactive biological function on a protein is generally understood in the art (Kyte and Doolittle, *J. Mol. Biol.* 157, 105-132 (1982)). It is accepted that the relative hydrophathic character of the amino acid contributes to the secondary structure of the resultant polypeptide, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

Each amino acid has been assigned a hydrophathic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, *J. Mol. Biol.* 157:105-132 (1982)); these are isoleucine (+4.5), valine (+4.2), leucine (+3.8), phenylalanine (+2.8), cysteine/cystine (+2.5), methionine (+1.9), alanine (+1.8), glycine (-0.4), threonine (-0.7), serine (-0.8), tryptophan (-0.9), tyrosine (-1.3), proline (-1.6), histidine (-3.2), glutamate (-3.5), glutamine (-3.5), aspartate (-3.5), asparagine (-3.5), lysine (-3.9), and arginine (-4.5).

In making such changes, the substitution of amino acids whose hydrophathic indices are within ± 2 is preferred, those that are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent 4,554,101 states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0), lysine (+3.0), aspartate (+3.0 \pm 1), glutamate (+3.0 \pm 1), serine (+0.3), asparagine (+0.2), glutamine (+0.2), glycine (0), threonine (-0.4), proline (-0.5 \pm 1), alanine (-0.5), histidine (-0.5), cysteine (-1.0), methionine (-1.3), valine (-1.5), leucine (-1.8), isoleucine (-1.8), tyrosine (-2.3), phenylalanine (-2.5), and tryptophan (-3.4).

In making such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those that are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

In a further aspect of the present invention, one or more of the nucleic acid molecules of the present invention differ in nucleic acid sequence from those for which a specific sequence is provided herein because one or more codons has been replaced with a codon that encodes a conservative substitution of the amino acid originally encoded.

5 Agents of the invention include nucleic acid molecules that encode at least about a contiguous 10 amino acid region of a polypeptide of the present invention, more preferably at least about a contiguous 25, 40, 50, 100, or 125 amino acid region of a polypeptide of the present invention.

10 In a preferred embodiment, any of the nucleic acid molecules of the present invention can be operably linked to a promoter region that functions in a plant cell to cause the production of an mRNA molecule, where the nucleic acid molecule that is linked to the promoter is heterologous with respect to that promoter. As used herein, "heterologous" means not naturally occurring together.

PROTEIN AND PEPTIDE MOLECULES

15 A class of agents includes one or more of the polypeptide molecules encoded by a nucleic acid agent of the invention. A particular preferred class of proteins is that having an amino acid sequence selected from the group consisting of SEQ ID NOs: 16 through 38, and fragments thereof. In a further aspect of the present invention the polypeptide molecule comprises an amino acid sequence selected from the group consisting of SEQ ID
20 NOs: 17 through 21, and fragments thereof. In a further aspect of the present invention the polypeptide molecule comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 22 through 27, and fragments thereof. In a further aspect of the present invention the polypeptide molecule comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 28 through 38, and fragments thereof. In a
25 further aspect of the present invention the polypeptide molecule comprises an amino acid sequence encoding an amino acid of SEQ ID NO: 28 and fragments thereof. In a further aspect of the present invention the polypeptide molecule comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 29 through 32, and fragments thereof. In a further aspect of the present invention the polypeptide molecule comprises an amino
30 acid sequence selected from the group consisting of SEQ ID NOs: 33 through 38, and fragments thereof.

In another embodiment, the present invention provides a polypeptide comprising the amino acid sequence of SEQ ID NO: 108. In another aspect, the present invention provides a polypeptide comprising the amino acid sequence of residues 83 through 356 of SEQ ID NO: 108. In another aspect, the present invention provides a polypeptide fragment
5 comprising the amino acid sequence of residues 83 through 356 of SEQ ID NO: 108, wherein the fragment has a length of at least about 25, 50, 75, 100, 150, 200, or 250 residues. In yet another aspect, the present invention provides a polypeptide comprising the amino acid sequence of one or more of the following fragments of SEQ ID NO: 108: 82 through 123, 132 through 146, and 269 through 295.

10 Polypeptide agents may have C-terminal or N-terminal amino acid sequence extensions. One class of N-terminal extensions employed in a preferred embodiment are plastid transit peptides. When employed, plastid transit peptides can be operatively linked to the N-terminal sequence, thereby permitting the localization of the agent polypeptides to plastids. In an embodiment of the present invention, any suitable plastid targeting
15 sequence can be used. Where suitable, a plastid targeting sequence can be substituted for a native plastid targeting sequence, for example, for the CTP occurring natively in the tMT2 protein. In a further embodiment, a plastid targeting sequence that is heterologous to any tMT2 protein or fragment described herein can be used. In a further embodiment, any suitable, modified plastid targeting sequence can be used. In another embodiment, the
20 plastid targeting sequence is a CTP1 sequence (*see* WO 00/61771).

In a preferred aspect a protein of the present invention is targeted to a plastid using either a native transit peptide sequence or a heterologous transit peptide sequence. In the case of nucleic acid sequences corresponding to nucleic acid sequences of non-higher plant organisms such as cyanobacteria, such nucleic acid sequences can be modified to attach the
25 coding sequence of the protein to a nucleic acid sequence of a plastid targeting peptide.

As used herein, the term "protein," "peptide molecule," or "polypeptide" includes any molecule that comprises five or more amino acids. It is well known in the art that protein, peptide or polypeptide molecules may undergo modification, including post-translational modifications, such as, but not limited to, disulfide bond formation,
30 glycosylation, phosphorylation, or oligomerization. Thus, as used herein, the term "protein," "peptide molecule," or "polypeptide" includes any protein that is modified by any biological or non-biological process. The terms "amino acid" and "amino acids" refer

to all naturally occurring L-amino acids. This definition is meant to include norleucine, norvaline, ornithine, homocysteine, and homoserine.

One or more of the protein or fragments thereof, peptide molecules, or polypeptide molecules may be produced via chemical synthesis, or more preferably, by expression in a suitable bacterial or eukaryotic host. Suitable methods for expression are described by
5 Sambrook *et al.*, In: *Molecular Cloning, A Laboratory Manual, 2nd Edition, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989)* or similar texts.

A “protein fragment” is a peptide or polypeptide molecule whose amino acid sequence comprises a subset of the amino acid sequence of that protein. A protein or
10 fragment thereof that comprises one or more additional peptide regions not derived from that protein is a “fusion” protein. Such molecules may be derivatized to contain carbohydrate or other moieties (such as keyhole limpet hemocyanin). Fusion protein or peptide molecules of the invention are preferably produced via recombinant means.

Another class of agents comprise protein, peptide molecules, or polypeptide molecules or fragments or fusions thereof comprising SEQ ID NOs: 16 through 38, and
15 fragments thereof in which conservative, non-essential or non-relevant amino acid residues have been added, replaced or deleted. Computerized means for designing modifications in protein structure are known in the art (Dahiyat and Mayo, *Science* 278:82-87 (1997)).

A protein, peptide or polypeptide of the invention can also be a homolog protein, peptide or polypeptide. As used herein, a homolog protein, peptide or polypeptide or
20 fragment thereof is a counterpart protein, peptide or polypeptide or fragment thereof in a second species. A homolog can also be generated by molecular evolution or DNA shuffling techniques, so that the molecule retains at least one functional or structure characteristic of the original (*see*, for example, U.S. Patent 5,811,238).

In another embodiment, the homolog is selected from the group consisting of alfalfa, *Arabidopsis*, barley, broccoli, cabbage, canola, citrus, cotton, garlic, oat, *Allium*,
25 flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, corn, and *Phaseolus*. More
30 particularly, preferred homologs are selected from canola, rapeseed, corn, *Brassica campestris*, *Brassica napus*, oilseed rape, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, safflower, oil palm, flax, and sunflower. In an even more

preferred embodiment, the homolog is selected from the group consisting of canola, rapeseed, corn, *Brassica campestris*, *Brassica napus*, oilseed rape, soybean, sunflower, safflower, oil palms, and peanut. In a preferred embodiment, the homolog is soybean. In a preferred embodiment, the homolog is canola. In a preferred embodiment, the homolog is oilseed rape.

In a preferred embodiment, the nucleic acid molecules of the present invention or complements and fragments of either can be utilized to obtain such homologs.

Agents of the invention include proteins and fragments thereof comprising at least about a contiguous 10 amino acid region preferably comprising at least about a contiguous 20 amino acid region, even more preferably comprising at least about a contiguous 25, 35, 50, 75 or 100 amino acid region of a protein of the present invention. In another preferred embodiment, the proteins of the present invention include between about 10 and about 25 contiguous amino acid region, more preferably between about 20 and about 50 contiguous amino acid region, and even more preferably between about 40 and about 80 contiguous amino acid region.

PLANT CONSTRUCTS AND PLANT TRANSFORMANTS

One or more of the nucleic acid molecules of the invention may be used in plant transformation or transfection. Exogenous genetic material may be transferred into a plant cell and the plant cell regenerated into a whole, fertile or sterile plant. Exogenous genetic material is any genetic material, whether naturally occurring or otherwise, from any source that is capable of being inserted into any organism.

In a preferred aspect of the present invention the exogenous genetic material comprises a nucleic acid sequence that encodes tocopherol methyltransferase. In another preferred aspect of the present invention the exogenous genetic material of the invention comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 through 15, and complements thereof and fragments of either. In a further aspect of the present invention the exogenous genetic material comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 16 through 38, and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 17 through 21, and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid

sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 22 through 27, and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 28 through 38, and fragments thereof.

5 In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid of SEQ ID NO: 28, and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 29 through 32, and fragments thereof. In a further aspect of the present invention the
10 nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 33 through 38, and fragments thereof. In a further aspect of the present invention, the nucleic acid sequences of the invention also encode peptides involved in intracellular localization, export, or post-translational modification.

15 In an embodiment of the present invention, exogenous genetic material comprising a tMT2 enzyme or fragment thereof is introduced into a plant with one or more additional genes. In one embodiment, preferred combinations of genes include one or more of the following genes: *tyrA*, *slr1736*, *HPT*, *GMT*, *tocopherol cyclase*, *dxs*, *dxr*, *GGPPS*, *HPPD*, *GMT*, *tMT2*, *AANT1*, *slr1737*, *IDI*, *GGH*, or a plant ortholog thereof, and an antisense
20 construct for homogentisic acid dioxygenase (Kridl *et al.*, *Seed Sci. Res.* 1:209:219 (1991); Keegstra, *Cell* 56(2):247-53 (1989); Nawrath, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 91:12760-12764 (1994); Xia *et al.*, *J. Gen. Microbiol.* 138:1309-1316 (1992); Cyanobase, www.kazusa.or.jp/cyanobase; Lois *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 95 (5):2105-2110 (1998); Takahashi *et al.* *Proc. Natl. Acad. Sci. U.S.A.* 95 (17), 9879-9884 (1998); Norris *et al.*, *Plant Physiol.* 117:1317-1323 (1998); Bartley and Scolnik, *Plant Physiol.* 104:1469-
25 1470 (1994), Smith *et al.*, *Plant J.* 11:83-92 (1997); WO 00/32757; WO 00/10380; Saint Guily, *et al.*, *Plant Physiol.*, 100(2):1069-1071 (1992); Sato *et al.*, *J. DNA Res.* 7 (1):31-63 (2000)).

In another preferred embodiment, tMT2 is combined with *GMT*. In any of the
30 embodiments disclosed herein in which a nucleic acid molecule encoding a *GMT* is used, the nucleic acid molecule is preferably selected from the group consisting of nucleic acid molecules comprising a nucleic acid sequence selected from the group SEQ ID NOs: 39 and 54, and nucleic acids molecules encoding GMTs having an amino acid sequence

selected from the group consisting of SEQ ID NOs: 39-54. In another preferred embodiment, tMT2 is combined with *GMT* and one or more of the genes listed above. In such combinations, one or more of the gene products can be directed to the plastid by the use of a plastid targeting sequence. Alternatively, one or more of the gene products can be localized in the cytoplasm. In a preferred aspect the gene products of *tyrA* and HPPD are targeted to the cytoplasm. Such genes can be introduced, for example, with the tMT2 or *GMT* or both, or fragment of either or both on a single construct, introduced on different constructs but the same transformation event, or introduced into separate plants followed by one or more crosses to generate the desired combination of genes. In such combinations, a preferred promoter is a napin promoter and a preferred plastid targeting sequence is a CTP1 sequence. It is preferred that gene products are targeted to the plastid.

In a preferred combination a nucleic acid molecule encoding a tMT2 polypeptide and a nucleic acid molecule encoding any of the following enzymes: *tyrA*, *slr1736*, *HPT*, *GMT*, *tocopherol cyclase*, *dxs*, *dxr*, *GGPPS*, *HPPD*, *tMT2*, *AANT1*, *slr1737*, *IDI*, *GGH* or a plant ortholog thereof, and an antisense construct for homogentisic acid dioxygenase are introduced into a plant. A particularly preferred combination that can be introduced is a nucleic acid molecule encoding a tMT2 polypeptide and a nucleic acid molecule encoding a *GMT* polypeptide, where both polypeptides are targeted to the plastid and where one of such polypeptides is present and the other is introduced. Both nucleic acid sequences encoding such polypeptides can be introduced using a single gene construct, or each polypeptide can be introduced on separate constructs. In a further embodiment, tMT2 is combined with *GMT* and one or more of *tyrA*, *slr1736*, *HPT*, *tocopherol cyclase*, *dxs*, *dxr*, *GGPPS*, *HPPD*, *AANT1*, *slr1737*, *IDI*, and *GGH*.

In a particularly preferred combination, a nucleic acid molecule encoding a tMT2 protein and a nucleic acid molecule encoding a *GMT* enzyme are introduced into a plant along with a nucleic acid molecule that encodes one or more of *tyrA*, *slr1736*, *HPT*, *tocopherol cyclase*, *dxs*, *dxr*, *GGPPS*, *HPPD*, *AANT1*, *slr1737*, *IDI*, and *GGH*.

Another particularly preferred combination that can be introduced is a nucleic acid molecule encoding a tMT2 protein and a nucleic acid molecule that results in the down regulation of a *GMT* protein. In such an aspect, it is preferred that the plant accumulates either γ -tocopherol or γ -tocotrienol or both.

Such genetic material may be transferred into either monocotyledons or dicotyledons including, but not limited to canola, corn, soybean, *Arabidopsis phaseolus*, peanut, alfalfa, wheat, rice, oat, sorghum, rapeseed, rye, tritordeum, millet, fescue, perennial ryegrass, sugarcane, cranberry, papaya, banana, safflower, oil palms, flax, muskmelon, apple, cucumber, dendrobium, gladiolus, chrysanthemum, liliacea, cotton, eucalyptus, sunflower, *Brassica campestris*, oilseed rape, turfgrass, sugarbeet, coffee and dioscorea (Christou, In: *Particle Bombardment for Genetic Engineering of Plants*, Biotechnology Intelligence Unit. Academic Press, San Diego, California (1996)), with canola, corn, *Brassica campestris*, *Brassica napus*, oilseed rape, rapeseed, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, safflower, oil palm, flax, and sunflower preferred, and canola, rapeseed, corn, *Brassica campestris*, *Brassica napus*, oilseed rape, soybean, sunflower, safflower, oil palms, and peanut preferred. In a more preferred embodiment, the genetic material is transferred into canola. In another more preferred embodiment, the genetic material is transferred into oilseed rape. In another particularly preferred embodiment, the genetic material is transferred into soybean.

Transfer of a nucleic acid molecule that encodes a protein can result in expression or overexpression of that polypeptide in a transformed cell or transgenic plant. One or more of the proteins or fragments thereof encoded by nucleic acid molecules of the invention may be overexpressed in a transformed cell or transformed plant. Such expression or overexpression may be the result of transient or stable transfer of the exogenous genetic material.

In a preferred embodiment, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of tocopherols.

In a preferred embodiment, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of α -tocopherols.

In a preferred embodiment, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of γ -tocopherols.

In a preferred embodiment, reduction of the expression, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant,

relative to an untransformed plant with a similar genetic background, an increased level of δ -tocopherols.

In a preferred embodiment, reduction of the expression, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant,
5 relative to an untransformed plant with a similar genetic background, an increased level of β -tocopherols.

In a preferred embodiment, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of tocotrienols.

10 In a preferred embodiment, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of α -tocotrienols.

In a preferred embodiment, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a
15 similar genetic background, an increased level of γ -tocotrienols.

In a preferred embodiment, reduction of the expression, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of δ -tocotrienols.

20 In a preferred embodiment, reduction of the expression, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of β -tocotrienols.

In a preferred embodiment, expression or overexpression of a polypeptide of the present invention in combination with a nucleic acid molecule encoding any of the
25 following enzymes: *tyrA*, *slr1736*, *HPT*, *GMT*, *tocopherol cyclase*, *dxs*, *dxr*, *GGPPS*, *HPPD*, *tMT2*, *AANT1*, *slr1737*, *IDI*, *GGH* or a plant ortholog thereof, and an antisense construct for homogentisic acid dioxygenase in a plant, provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of total
30 tocopherols.

In a preferred embodiment, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of plastoquinols.

5 In a preferred embodiment, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of total tocopherols.

In any of the embodiments described herein, an increase in γ -tocopherol, α -tocopherol, or both can lead to a decrease in the relative proportion of β -tocopherol, δ -tocopherol, or both. Similarly, an increase in γ -tocotrienol, α -tocotrienol, or both can lead
10 to a decrease in the relative proportion of β -tocotrienol, δ -tocotrienol, or both.

In another embodiment, expression, overexpression of a polypeptide of the present invention in a plant provides in that plant, or a tissue of that plant, relative to an untransformed plant or plant tissue, with a similar genetic background, an increased level of a tMT2 protein or fragment thereof.

15 In some embodiments, the levels of one or more products of the tocopherol biosynthesis pathway, including any one or more of tocopherols, α -tocopherols, γ -tocopherols, δ -tocopherols, β -tocopherols, tocotrienols, α -tocotrienols, γ -tocotrienols, δ -tocotrienols, β -tocotrienols are increased by greater than about 10%, or more preferably greater than about 25%, 35%, 50%, 75%, 80%, 90%, 100%, 150%, 200%, 1,000%,
20 2,000%, or 2,500%. The levels of products may be increased throughout an organism such as a plant or localized in one or more specific organs or tissues of the organism. For example the levels of products may be increased in one or more of the tissues and organs of a plant including without limitation: roots, tubers, stems, leaves, stalks, fruit, berries, nuts, bark, pods, seeds and flowers. A preferred organ is a seed.

25 In some embodiments, the levels of one or more products of the tocopherol biosynthesis pathway, including any one or more of tocopherols, α -tocopherols, γ -tocopherols, δ -tocopherols, β -tocopherols, tocotrienols, α -tocotrienols, γ -tocotrienols, δ -tocotrienols, β -tocotrienols are increased so that they constitute greater than about 10%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%,
30 95%, 96%, 97%, 98%, or 99% of the total tocopherol content of the organism or tissue. The levels of products may be increased throughout an organism such as a plant or

localized in one or more specific organs or tissues of the organism. For example the levels of products may be increased in one or more of the tissues and organs of a plant including without limitation: roots, tubers, stems, leaves, stalks, fruit, berries, nuts, bark, pods, seeds and flowers. A preferred organ is a seed.

5 In a preferred embodiment, expression of enzymes involved in tocopherol, tocotrienol or plastoquinol synthesis in the seed will result in an increase in γ -tocopherol levels due to the absence of significant levels of GMT activity in those tissues. In another preferred embodiment, expression of enzymes involved in tocopherol, tocotrienol or plastoquinol synthesis in photosynthetic tissues will result in an increase in α -tocopherol
10 due to the higher levels of GMT activity in those tissues relative to the same activity in seed tissue.

 In another preferred embodiment, the expression of enzymes involved in tocopherol, tocotrienol or plastoquinol synthesis in the seed will result in an increase in the total tocopherol, tocotrienol or plastoquinol level in the plant.

15 In some embodiments, the levels of tocopherols or a species such as α -tocopherol may be altered. In some embodiments, the levels of tocotrienols may be altered. Such alteration can be compared to a plant with a similar background.

 In another embodiment, either the α -tocopherol level, α -tocotrienol level, or both of plants that natively produce high levels of either α -tocopherol, α -tocotrienol or both (*e.g.*,
20 sunflowers), can be increased by the introduction of a gene coding for a tMT2 enzyme.

 In a preferred aspect, a similar genetic background is a background where the organisms being compared share about 50% or greater of their nuclear genetic material. In a more preferred aspect a similar genetic background is a background where the organisms being compared share about 75% or greater, even more preferably about 90% or greater of
25 their nuclear genetic material. In another even more preferable aspect, a similar genetic background is a background where the organisms being compared are plants, and the plants are isogenic except for any genetic material originally introduced using plant transformation techniques.

 In another preferred embodiment, reduction of the expression, expression,
30 overexpression of a polypeptide of the present invention in a transformed plant may provide tolerance to a variety of stress, *e.g.* oxidative stress tolerance such as to oxygen or ozone, UV tolerance, cold tolerance, or fungal/microbial pathogen tolerance.

As used herein in a preferred aspect, a tolerance or resistance to stress is determined by the ability of a plant, when challenged by a stress such as cold to produce a plant having a higher yield than one without such tolerance or resistance to stress. In a particularly preferred aspect of the present invention, the tolerance or resistance to stress is measured relative to a plant with a similar genetic background to the tolerant or resistance plant except that the plant reduces the expression, expresses or over expresses a protein or fragment thereof of the present invention.

Exogenous genetic material may be transferred into a host cell by the use of a DNA vector or construct designed for such a purpose. Design of such a vector is generally within the skill of the art (*See, Plant Molecular Biology: A Laboratory Manual*, Clark (ed.), Springer, New York (1997)).

A construct or vector may include a plant promoter to express the polypeptide of choice. In a preferred embodiment, any nucleic acid molecules described herein can be operably linked to a promoter region which functions in a plant cell to cause the production of an mRNA molecule. For example, any promoter that functions in a plant cell to cause the production of an mRNA molecule, such as those promoters described herein, without limitation, can be used. In a preferred embodiment, the promoter is a plant promoter.

A number of promoters that are active in plant cells have been described in the literature. These include the nopaline synthase (NOS) promoter (Ebert *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 84:5745-5749 (1987)), the octopine synthase (OCS) promoter (which is carried on tumor-inducing plasmids of *Agrobacterium tumefaciens*), the caulimovirus promoters such as the cauliflower mosaic virus (CaMV) 19S promoter (Lawton *et al.*, *Plant Mol. Biol.* 9:315-324 (1987)) and the CaMV 35S promoter (Odell *et al.*, *Nature* 313:810-812 (1985)), the figwort mosaic virus 35S-promoter, the light-inducible promoter from the small subunit of ribulose-1,5-bis-phosphate carboxylase (ssRUBISCO), the Adh promoter (Walker *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 84:6624-6628 (1987)), the sucrose synthase promoter (Yang *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 87:4144-4148 (1990)), the R gene complex promoter (Chandler *et al.*, *The Plant Cell* 1:1175-1183 (1989)) and the chlorophyll a/b binding protein gene promoter, *etc.* These promoters have been used to create DNA constructs that have been expressed in plants; *see, e.g.*, PCT publication WO 84/02913. The CaMV 35S promoters are preferred for use in plants. Promoters known or found to cause transcription of DNA in plant cells can be used in the invention.

For the purpose of expression in source tissues of the plant, such as the leaf, seed, root or stem, it is preferred that the promoters utilized have relatively high expression in these specific tissues. Tissue-specific expression of a protein of the present invention is a particularly preferred embodiment. For this purpose, one may choose from a number of promoters for genes with tissue- or cell-specific or enhanced expression. Examples of such promoters reported in the literature include the chloroplast glutamine synthetase GS2 promoter from pea (Edwards *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 87:3459-3463 (1990)), the chloroplast fructose-1,6-biphosphatase (FBPase) promoter from wheat (Lloyd *et al.*, *Mol. Gen. Genet.* 225:209-216 (1991)), the nuclear photosynthetic ST-LS1 promoter from potato (Stockhaus *et al.*, *EMBO J.* 8:2445-2451 (1989)), the serine/threonine kinase (PAL) promoter and the glucoamylase (CHS) promoter from *Arabidopsis thaliana*. Also reported to be active in photosynthetically active tissues are the ribulose-1,5-bisphosphate carboxylase (RbcS) promoter from eastern larch (*Larix laricina*), the promoter for the *cab* gene, *cab6*, from pine (Yamamoto *et al.*, *Plant Cell Physiol.* 35:773-778 (1994)), the promoter for the Cab-1 gene from wheat (Fejes *et al.*, *Plant Mol. Biol.* 15:921-932 (1990)), the promoter for the CAB-1 gene from spinach (Lubberstedt *et al.*, *Plant Physiol.* 104:997-1006 (1994)), the promoter for the *cab1R* gene from rice (Luan *et al.*, *Plant Cell.* 4:971-981 (1992)), the pyruvate, orthophosphate dikinase (PPDK) promoter from corn (Matsuoka *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 90:9586-9590 (1993)), the promoter for the tobacco *Lhcb1*2* gene (Cerdan *et al.*, *Plant Mol. Biol.* 33:245-255 (1997)), the *Arabidopsis thaliana* SUC2 sucrose-H⁺ symporter promoter (Truernit *et al.*, *Planta.* 196:564-570 (1995)) and the promoter for the thylakoid membrane proteins from spinach (*psaD*, *psaF*, *psaE*, PC, FNR, *atpC*, *atpD*, *cab*, *rbcS*). Other promoters for the chlorophyll a/b-binding proteins may also be utilized in the invention, such as the promoters for *Lhcb* gene and *PsbP* gene from white mustard (*Sinapis alba*; Kretsch *et al.*, *Plant Mol. Biol.* 28:219-229 (1995)).

For the purpose of expression in sink tissues of the plant, such as the tuber of the potato plant, the fruit of tomato, or the seed of corn, wheat, rice and barley, it is preferred that the promoters utilized in the invention have relatively high expression in these specific tissues. A number of promoters for genes with tuber-specific or tuber-enhanced expression are known, including the class I patatin promoter (Bevan *et al.*, *EMBO J.* 8:1899-1906 (1986); Jefferson *et al.*, *Plant Mol. Biol.* 14:995-1006 (1990)), the promoter for the potato tuber ADPGPP genes, both the large and small subunits, the sucrose synthase promoter

(Salanoubat and Belliard, *Gene* 60:47-56 (1987), Salanoubat and Belliard, *Gene* 84:181-185 (1989)), the promoter for the major tuber proteins including the 22 kd protein complexes and protease inhibitors (Hannapel, *Plant Physiol.* 101:703-704 (1993)), the promoter for the granule-bound starch synthase gene (GBSS) (Visser *et al.*, *Plant Mol. Biol.* 17:691-699 (1991)) and other class I and II patatins promoters (Koster-Topfer *et al.*, *Mol. Gen. Genet.* 219:390-396 (1989); Mignery *et al.*, *Gene*. 62:27-44 (1988)).

Other promoters can also be used to express a polypeptide in specific tissues, such as seeds or fruits. Indeed, in a preferred embodiment, the promoter used is a seed specific promoter. Examples of such promoters include the 5' regulatory regions from such genes as napin (Kridl *et al.*, *Seed Sci. Res.* 1:209:219 (1991)), phaseolin (Bustos, *et al.*, *Plant Cell*, 1(9):839-853 (1989)), soybean trypsin inhibitor (Riggs, *et al.*, *Plant Cell* 1(6):609-621 (1989)), ACP (Baerson, *et al.*, *Plant Mol. Biol.*, 22(2):255-267 (1993)), stearyl-ACP desaturase (Slocombe, *et al.*, *Plant Physiol.* 104(4):167-176 (1994)), soybean a' subunit of b-conglycinin (soy 7s, (Chen *et al.*, *Proc. Natl. Acad. Sci.*, 83:8560-8564 (1986))), and oleosin (see, for example, Hong, *et al.*, *Plant Mol. Biol.*, 34(3):549-555 (1997)). Further examples include the promoter for β -conglycinin (Chen *et al.*, *Dev. Genet.* 10:112-122 (1989)). Also included are the zeins, which are a group of storage proteins found in corn endosperm. Genomic clones for zein genes have been isolated (Pedersen *et al.*, *Cell* 29:1015-1026 (1982), and Russell *et al.*, *Transgenic Res.* 6(2):157-168) and the promoters from these clones, including the 15 kD, 16 kD, 19 kD, 22 kD, 27 kD and genes, could also be used. Other promoters known to function, for example, in corn include the promoters for the following genes: *waxy*, *Brittle*, *Shrunken 2*, Branching enzymes I and II, starch synthases, debranching enzymes, oleosins, glutelins and sucrose synthases. A particularly preferred promoter for corn endosperm expression is the promoter for the glutelin gene from rice, more particularly the Osgt-1 promoter (Zheng *et al.*, *Mol. Cell Biol.* 13:5829-5842 (1993)). Examples of promoters suitable for expression in wheat include those promoters for the ADPGlucose pyrosynthase (ADPGPP) subunits, the granule bound and other starch synthase, the branching and debranching enzymes, the embryogenesis-abundant proteins, the gliadins and the glutenins. Examples of such promoters in rice include those promoters for the ADPGPP subunits, the granule bound and other starch synthase, the branching enzymes, the debranching enzymes, sucrose synthases and the glutelins. A particularly preferred promoter is the promoter for rice glutelin, Osgt-1. Examples of such promoters for barley include those for the ADPGPP subunits, the granule

bound and other starch synthase, the branching enzymes, the debranching enzymes, sucrose synthases, the hordeins, the embryo globulins and the aleurone specific proteins. A preferred promoter for expression in the seed is a napin promoter. Another preferred promoter for expression is an Arcelin5 promoter.

5 Root specific promoters may also be used. An example of such a promoter is the promoter for the acid chitinase gene (Samac *et al.*, *Plant Mol. Biol.* 25:587-596 (1994)). Expression in root tissue could also be accomplished by utilizing the root specific subdomains of the CaMV35S promoter that have been identified (Lam *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:7890-7894 (1989)). Other root cell specific promoters include those
10 reported by Conkling *et al.* (Conkling *et al.*, *Plant Physiol.* 93:1203-1211 (1990)).

Additional promoters that may be utilized are described, for example, in U.S. Patents 5,378,619; 5,391,725; 5,428,147; 5,447,858; 5,608,144; 5,608,144; 5,614,399; 5,633,441; 5,633,435; and 4,633,436. In addition, a tissue specific enhancer may be used (Fromm *et al.*, *The Plant Cell* 1:977-984 (1989)).

15 Constructs or vectors may also include, with the coding region of interest, a nucleic acid sequence that acts, in whole or in part, to terminate transcription of that region. A number of such sequences have been isolated, including the Tr7 3' sequence and the NOS 3' sequence (Ingelbrecht *et al.*, *The Plant Cell* 1:671-680 (1989); Bevan *et al.*, *Nucleic Acids Res.* 11:369-385 (1983)). Regulatory transcript termination regions can be provided
20 in plant expression constructs of this invention as well. Transcript termination regions can be provided by the DNA sequence encoding the gene of interest or a convenient transcription termination region derived from a different gene source, for example, the transcript termination region that is naturally associated with the transcript initiation region. The skilled artisan will recognize that any convenient transcript termination region that is
25 capable of terminating transcription in a plant cell can be employed in the constructs of the present invention.

A vector or construct may also include regulatory elements. Examples of such include the Adh intron 1 (Callis *et al.*, *Genes and Develop.* 1:1183-1200 (1987)), the sucrose synthase intron (Vasil *et al.*, *Plant Physiol.* 91:1575-1579 (1989)) and the TMV
30 omega element (Gallie *et al.*, *The Plant Cell* 1:301-311 (1989)). These and other regulatory elements may be included when appropriate.

A vector or construct may also include a selectable marker. Selectable markers may also be used to select for plants or plant cells that contain the exogenous genetic material. Examples of such include, but are not limited to: a *neo* gene (Potrykus *et al.*, *Mol. Gen. Genet.* 199:183-188 (1985)), which codes for kanamycin resistance and can be
5 selected for using kanamycin, RptII, G418, hpt *etc.*; a bar gene which codes for bialaphos resistance; a mutant EPSP synthase gene (Hinchee *et al.*, *Bio/Technology* 6:915-922 (1988); Reynaerts *et al.*, *Selectable and Screenable Markers*. In Gelvin and Schilperoort. *Plant Molecular Biology Manual*, Kluwer, Dordrecht (1988); Reynaerts *et al.*, *Selectable and screenable markers*. In Gelvin and Schilperoort. *Plant Molecular Biology Manual*,
10 Kluwer, Dordrecht (1988)), *aadA* (Jones *et al.*, *Mol. Gen. Genet.* (1987)), which encodes glyphosate resistance; a nitrilase gene which confers resistance to bromoxynil (Stalker *et al.*, *J. Biol. Chem.* 263:6310-6314 (1988)); a mutant acetolactate synthase gene (ALS) which confers imidazolinone or sulphonylurea resistance (European Patent Application 154,204 (Sept. 11, 1985)), ALS (D'Halluin *et al.*, *Bio/Technology* 10:309-314 (1992)), and
15 a methotrexate resistant DHFR gene (Thillet *et al.*, *J. Biol. Chem.* 263:12500-12508 (1988)).

A vector or construct may also include a transit peptide. Incorporation of a suitable chloroplast transit peptide may also be employed (European Patent Application Publication Number 0218571). Translational enhancers may also be incorporated as part of the vector
20 DNA. DNA constructs could contain one or more 5' non-translated leader sequences, which may serve to enhance expression of the gene products from the resulting mRNA transcripts. Such sequences may be derived from the promoter selected to express the gene or can be specifically modified to increase translation of the mRNA. Such regions may also be obtained from viral RNAs, from suitable eukaryotic genes, or from a synthetic gene
25 sequence. For a review of optimizing expression of transgenes, see Koziel *et al.*, *Plant Mol. Biol.* 32:393-405 (1996). A preferred transit peptide is CTP1.

A vector or construct may also include a screenable marker. Screenable markers may be used to monitor expression. Exemplary screenable markers include: a β -glucuronidase or *uidA* gene (GUS) which encodes an enzyme for which various
30 chromogenic substrates are known (Jefferson, *Plant Mol. Biol., Rep.* 5:387-405 (1987); Jefferson *et al.*, *EMBO J.* 6:3901-3907 (1987)); an R-locus gene, which encodes a product that regulates the production of anthocyanin pigments (red color) in plant tissues (Dellaporta *et al.*, *Stadler Symposium* 11:263-282 (1988)); a β -lactamase gene (Sutcliffe *et*

al., *Proc. Natl. Acad. Sci. (U.S.A.)* 75:3737-3741 (1978)), a gene which encodes an enzyme for which various chromogenic substrates are known (*e.g.*, PADAC, a chromogenic cephalosporin); a luciferase gene (Ow *et al.*, *Science* 234:856-859 (1986)); a *xyIE* gene (Zukowsky *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 80:1101-1105 (1983)) which encodes a catechol dioxygenase that can convert chromogenic catechols; an α -amylase gene (Ikatsu *et al.*, *Bio/Technol.* 8:241-242 (1990)); a tyrosinase gene (Katz *et al.*, *J. Gen. Microbiol.* 129:2703-2714 (1983)) which encodes an enzyme capable of oxidizing tyrosine to DOPA and dopaquinone which in turn condenses to melanin; an α -galactosidase, which will turn a chromogenic α -galactose substrate.

10 Included within the terms "selectable or screenable marker genes" are also genes that encode a secretable marker whose secretion can be detected as a means of identifying or selecting for transformed cells. Examples include markers that encode a secretable antigen that can be identified by antibody interaction, or even secretable enzymes that can be detected catalytically. Secretable proteins fall into a number of classes, including small, 15 diffusible proteins that are detectable, (*e.g.*, by ELISA), small active enzymes that are detectable in extracellular solution (*e.g.*, α -amylase, β -lactamase, phosphinothricin transferase), or proteins that are inserted or trapped in the cell wall (such as proteins that include a leader sequence such as that found in the expression unit of extension or tobacco PR-S). Other possible selectable and/or screenable marker genes will be apparent to those 20 of skill in the art.

There are many methods for introducing transforming nucleic acid molecules into plant cells. Suitable methods are believed to include virtually any method by which nucleic acid molecules may be introduced into a cell, such as by *Agrobacterium* infection or direct delivery of nucleic acid molecules such as, for example, by PEG-mediated 25 transformation, by electroporation or by acceleration of DNA coated particles, and the like. (Potrykus, *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 42:205-225 (1991); Vasil, *Plant Mol. Biol.* 25:925-937 (1994)). For example, electroporation has been used to transform corn protoplasts (Fromm *et al.*, *Nature* 312:791-793 (1986)).

Other vector systems suitable for introducing transforming DNA into a host plant 30 cell include but are not limited to binary artificial chromosome (BIBAC) vectors (Hamilton *et al.*, *Gene* 200:107-116 (1997)); and transfection with RNA viral vectors (Della-Cioppa *et al.*, *Ann. N.Y. Acad. Sci.* (1996), 792 (Engineering Plants for Commercial Products and

Applications), 57-61). Additional vector systems also include plant selectable YAC vectors such as those described in Mullen *et al.*, *Molecular Breeding* 4:449-457 (1988).

Technology for introduction of DNA into cells is well known to those of skill in the art. Four general methods for delivering a gene into cells have been described:

- 5 (1) chemical methods (Graham and van der Eb, *Virology* 54:536-539 (1973)); (2) physical methods such as microinjection (Capecchi, *Cell* 22:479-488 (1980)), electroporation (Wong and Neumann, *Biochem. Biophys. Res. Commun.* 107:584-587 (1982); Fromm *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 82:5824-5828 (1985); U.S. Patent 5,384,253); the gene gun (Johnston and Tang, *Methods Cell Biol.* 43:353-365 (1994)); and vacuum infiltration
- 10 (Bechtold *et al.*, *C.R. Acad. Sci. Paris, Life Sci.* 316:1194-1199. (1993)); (3) viral vectors (Clapp, *Clin. Perinatol.* 20:155-168 (1993); Lu *et al.*, *J. Exp. Med.* 178:2089-2096 (1993); Eglitis and Anderson, *Biotechniques* 6:608-614 (1988)); and (4) receptor-mediated mechanisms (Curiel *et al.*, *Hum. Gen. Ther.* 3:147-154 (1992), Wagner *et al.*, *Proc. Natl. Acad. Sci. (USA)* 89:6099-6103 (1992)).

- 15 Acceleration methods that may be used include, for example, microprojectile bombardment and the like. One example of a method for delivering transforming nucleic acid molecules into plant cells is microprojectile bombardment. This method has been reviewed by Yang and Christou (eds.), *Particle Bombardment Technology for Gene Transfer*, Oxford Press, Oxford, England (1994)). Non-biological particles
- 20 (microprojectiles) may be coated with nucleic acids and delivered into cells by a propelling force. Exemplary particles include those comprised of tungsten, gold, platinum and the like.

- A particular advantage of microprojectile bombardment, in addition to it being an effective means of reproducibly transforming monocots, is that neither the isolation of
- 25 protoplasts (Cristou *et al.*, *Plant Physiol.* 87:671-674 (1988)) nor the susceptibility to *Agrobacterium* infection is required. An illustrative embodiment of a method for delivering DNA into corn cells by acceleration is a biolistics α -particle delivery system, which can be used to propel particles coated with DNA through a screen, such as a stainless steel or Nytex screen, onto a filter surface covered with corn cells cultured in
 - 30 suspension. Gordon-Kamm *et al.*, describes the basic procedure for coating tungsten particles with DNA (Gordon-Kamm *et al.*, *Plant Cell* 2:603-618 (1990)). The screen disperses the tungsten nucleic acid particles so that they are not delivered to the recipient cells in large aggregates. A particle delivery system suitable for use with the invention is

the helium acceleration PDS-1000/He gun, which is available from Bio-Rad Laboratories (Bio-Rad, Hercules, California)(Sanford *et al.*, *Technique* 3:3-16 (1991)).

For the bombardment, cells in suspension may be concentrated on filters. Filters containing the cells to be bombarded are positioned at an appropriate distance below the microprojectile stopping plate. If desired, one or more screens are also positioned between the gun and the cells to be bombarded.

Alternatively, immature embryos or other target cells may be arranged on solid culture medium. The cells to be bombarded are positioned at an appropriate distance below the microprojectile stopping plate. If desired, one or more screens are also positioned between the acceleration device and the cells to be bombarded. Through the use of techniques set forth herein one may obtain 1000 or more loci of cells transiently expressing a marker gene. The number of cells in a focus that express the exogenous gene product 48 hours post-bombardment often ranges from one to ten, and average one to three.

In bombardment transformation, one may optimize the pre-bombardment culturing conditions and the bombardment parameters to yield the maximum numbers of stable transformants. Both the physical and biological parameters for bombardment are important in this technology. Physical factors are those that involve manipulating the DNA/microprojectile precipitate or those that affect the flight and velocity of either the macro- or microprojectiles. Biological factors include all steps involved in manipulation of cells before and immediately after bombardment, the osmotic adjustment of target cells to help alleviate the trauma associated with bombardment and also the nature of the transforming DNA, such as linearized DNA or intact supercoiled plasmids. It is believed that pre-bombardment manipulations are especially important for successful transformation of immature embryos.

In another alternative embodiment, plastids can be stably transformed. Methods disclosed for plastid transformation in higher plants include the particle gun delivery of DNA containing a selectable marker and targeting of the DNA to the plastid genome through homologous recombination (Svab *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 87:8526-8530 (1990); Svab and Maliga, *Proc. Natl. Acad. Sci. (U.S.A.)* 90:913-917 (1993); Staub and Maliga, *EMBO J.* 12:601-606 (1993); U.S. Patents 5,451,513 and 5,545,818).

Accordingly, it is contemplated that one may wish to adjust various aspects of the bombardment parameters in small scale studies to fully optimize the conditions. One may

particularly wish to adjust physical parameters such as gap distance, flight distance, tissue distance and helium pressure. One may also minimize the trauma reduction factors by modifying conditions that influence the physiological state of the recipient cells and which may therefore influence transformation and integration efficiencies. For example, the osmotic state, tissue hydration and the subculture stage or cell cycle of the recipient cells may be adjusted for optimum transformation. The execution of other routine adjustments will be known to those of skill in the art in light of the present disclosure.

Agrobacterium-mediated transfer is a widely applicable system for introducing genes into plant cells because the DNA can be introduced into whole plant tissues, thereby bypassing the need for regeneration of an intact plant from a protoplast. The use of *Agrobacterium*-mediated plant integrating vectors to introduce DNA into plant cells is well known in the art. See, for example the methods described by Fraley *et al.*, *Bio/Technology* 3:629-635 (1985) and Rogers *et al.*, *Methods Enzymol.* 153:253-277 (1987). Further, the integration of the Ti-DNA is a relatively precise process resulting in few rearrangements. The region of DNA to be transferred is defined by the border sequences and intervening DNA is usually inserted into the plant genome as described (Spielmann *et al.*, *Mol. Gen. Genet.* 205:34 (1986)).

Modern *Agrobacterium* transformation vectors are capable of replication in *E. coli* as well as *Agrobacterium*, allowing for convenient manipulations as described (Klee *et al.*, In: *Plant DNA Infectious Agents*, Hohn and Schell (eds.), Springer-Verlag, New York, pp. 179-203 (1985)). Moreover, technological advances in vectors for *Agrobacterium*-mediated gene transfer have improved the arrangement of genes and restriction sites in the vectors to facilitate construction of vectors capable of expressing various polypeptide coding genes. The vectors described have convenient multi-linker regions flanked by a promoter and a polyadenylation site for direct expression of inserted polypeptide coding genes and are suitable for present purposes (Rogers *et al.*, *Methods Enzymol.* 153:253-277 (1987)). In addition, *Agrobacterium* containing both armed and disarmed Ti genes can be used for the transformations. In those plant strains where *Agrobacterium*-mediated transformation is efficient, it is the method of choice because of the facile and defined nature of the gene transfer.

A transgenic plant formed using *Agrobacterium* transformation methods typically contains a single gene on one chromosome. Such transgenic plants can be referred to as being heterozygous for the added gene. More preferred is a transgenic plant that is

homozygous for the added structural gene; *i.e.*, a transgenic plant that contains two added genes, one gene at the same locus on each chromosome of a chromosome pair. A homozygous transgenic plant can be obtained by sexually mating (selfing) an independent segregant, transgenic plant that contains a single added gene, germinating some of the seed
5 produced and analyzing the resulting plants produced for the gene of interest.

It is also to be understood that two different transgenic plants can also be mated to produce offspring that contain two independently segregating, exogenous genes. Selfing of appropriate progeny can produce plants that are homozygous for both added, exogenous genes that encode a polypeptide of interest. Back-crossing to a parental plant and out-
10 crossing with a non-transgenic plant are also contemplated, as is vegetative propagation.

Transformation of plant protoplasts can be achieved using methods based on calcium phosphate precipitation, polyethylene glycol treatment, electroporation and combinations of these treatments (*See, for example, Potrykus et al., Mol. Gen. Genet.* 205:193-200 (1986); Lorz *et al., Mol. Gen. Genet.* 199:178 (1985); Fromm *et al., Nature*
15 319:791 (1986); Uchimiya *et al., Mol. Gen. Genet.* 204:204 (1986); Marcotte *et al., Nature* 335:454-457 (1988)).

Application of these systems to different plant strains depends upon the ability to regenerate that particular plant strain from protoplasts. Illustrative methods for the regeneration of cereals from protoplasts are described (Fujimura *et al., Plant Tissue*
20 *Culture Letters* 2:74 (1985); Toriyama *et al., Theor. Appl. Genet.* 205:34 (1986); Yamada *et al., Plant Cell Rep.* 4:85 (1986); Abdullah *et al., Biotechnology* 4:1087 (1986)).

To transform plant strains that cannot be successfully regenerated from protoplasts, other ways to introduce DNA into intact cells or tissues can be utilized. For example, regeneration of cereals from immature embryos or explants can be effected as described
25 (Vasil, *Biotechnology* 6:397 (1988)). In addition, "particle gun" or high-velocity microprojectile technology can be utilized (Vasil *et al., Bio/Technology* 10:667 (1992)).

Using the latter technology, DNA is carried through the cell wall and into the cytoplasm on the surface of small metal particles as described (Klein *et al., Nature* 328:70 (1987); Klein *et al., Proc. Natl. Acad. Sci. (U.S.A.)* 85:8502-8505 (1988); McCabe *et al.,*
30 *Bio/Technology* 6:923 (1988)). The metal particles penetrate through several layers of cells and thus allow the transformation of cells within tissue explants.

Other methods of cell transformation can also be used and include but are not limited to introduction of DNA into plants by direct DNA transfer into pollen (Hess *et al.*, *Intern Rev. Cytol.* 107:367 (1987); Luo *et al.*, *Plant Mol Biol. Reporter* 6:165 (1988)), by direct injection of DNA into reproductive organs of a plant (Pena *et al.*, *Nature* 325:274 (1987)), or by direct injection of DNA into the cells of immature embryos followed by the rehydration of desiccated embryos (Neuhaus *et al.*, *Theor. Appl. Genet.* 75:30 (1987)).

The regeneration, development and cultivation of plants from single plant protoplast transformants or from various transformed explants is well known in the art (Weissbach and Weissbach, In: *Methods for Plant Molecular Biology*, Academic Press, San Diego, CA, (1988)). This regeneration and growth process typically includes the steps of selection of transformed cells, culturing those individualized cells through the usual stages of embryonic development through the rooted plantlet stage. Transgenic embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth medium such as soil.

The development or regeneration of plants containing the foreign, exogenous gene that encodes a protein of interest is well known in the art. Preferably, the regenerated plants are self-pollinated to provide homozygous transgenic plants. Otherwise, pollen obtained from the regenerated plants is crossed to seed-grown plants of agronomically important lines. Conversely, pollen from plants of these important lines is used to pollinate regenerated plants. A transgenic plant of the invention containing a desired polypeptide is cultivated using methods well known to one skilled in the art.

There are a variety of methods for the regeneration of plants from plant tissue. The particular method of regeneration will depend on the starting plant tissue and the particular plant species to be regenerated.

Methods for transforming dicots, primarily by use of *Agrobacterium tumefaciens* and obtaining transgenic plants have been published for cotton (U.S. Patent 5,004,863; U.S. Patent 5,159,135; U.S. Patent 5,518,908); soybean (U.S. Patent 5,569,834; U.S. Patent 5,416,011; McCabe *et al.*, *Biotechnology* 6:923 (1988); Christou *et al.*, *Plant Physiol.* 87:671-674 (1988)); *Brassica* (U.S. Patent 5,463,174); peanut (Cheng *et al.*, *Plant Cell Rep.* 15:653-657 (1996), McKently *et al.*, *Plant Cell Rep.* 14:699-703 (1995)); papaya; pea (Grant *et al.*, *Plant Cell Rep.* 15:254-258 (1995)); and *Arabidopsis thaliana* (Bechtold *et al.*, *C.R. Acad. Sci. Paris, Life Sci.* 316:1194-1199 (1993)). The latter method for

transforming *Arabidopsis thaliana* is commonly called "dipping" or vacuum infiltration or germplasm transformation.

Transformation of monocotyledons using electroporation, particle bombardment and *Agrobacterium* have also been reported. Transformation and plant regeneration have been achieved in asparagus (Bytebier *et al.*, *Proc. Natl. Acad. Sci. (USA)* 84:5354 (1987));
5 barley (Wan and Lemaux, *Plant Physiol* 104:37 (1994)); corn (Rhodes *et al.*, *Science* 240:204 (1988); Gordon-Kamm *et al.*, *Plant Cell* 2:603-618 (1990); Fromm *et al.*, *Bio/Technology* 8:833 (1990); Koziel *et al.*, *Bio/Technology* 11:194 (1993); Armstrong *et al.*, *Crop Science* 35:550-557 (1995)); oat (Somers *et al.*, *Bio/Technology* 10:1589 (1992));
10 orchard grass (Horn *et al.*, *Plant Cell Rep.* 7:469 (1988)); rice (Toriyama *et al.*, *Theor Appl. Genet.* 205:34 (1986); Part *et al.*, *Plant Mol. Biol.* 32:1135-1148 (1996); Abedinia *et al.*, *Aust. J. Plant Physiol.* 24:133-141 (1997); Zhang and Wu, *Theor. Appl. Genet.* 76:835 (1988); Zhang *et al.*, *Plant Cell Rep.* 7:379 (1988); Battraw and Hall, *Plant Sci.* 86:191-202 (1992); Christou *et al.*, *Bio/Technology* 9:957 (1991)); rye (De la Pena *et al.*, *Nature*
15 325:274 (1987)); sugarcane (Bower and Birch, *Plant J.* 2:409 (1992)); tall fescue (Wang *et al.*, *Bio/Technology* 10:691 (1992)) and wheat (Vasil *et al.*, *Bio/Technology* 10:667 (1992); U.S. Patent 5,631,152).

Assays for gene expression based on the transient expression of cloned nucleic acid constructs have been developed by introducing the nucleic acid molecules into plant cells
20 by polyethylene glycol treatment, electroporation, or particle bombardment (Marcotte *et al.*, *Nature* 335:454-457 (1988); Marcotte *et al.*, *Plant Cell* 1:523-532 (1989); McCarty *et al.*, *Cell* 66:895-905 (1991); Hattori *et al.*, *Genes Dev.* 6:609-618 (1992); Goff *et al.*, *EMBO J.* 9:2517-2522 (1990)). Transient expression systems may be used to functionally dissect gene constructs (*see generally*, Mailga *et al.*, *Methods in Plant Molecular Biology*,
25 Cold Spring Harbor Press (1995)).

Any of the nucleic acid molecules of the invention may be introduced into a plant cell in a permanent or transient manner in combination with other genetic elements such as vectors, promoters, enhancers, *etc.* Further, any of the nucleic acid molecules of the invention may be introduced into a plant cell in a manner that allows for expression or
30 overexpression of the protein or fragment thereof encoded by the nucleic acid molecule.

Cosuppression is the reduction in expression levels, usually at the level of RNA, of a particular endogenous gene or gene family by the expression of a homologous sense

construct that is capable of transcribing mRNA of the same strandedness as the transcript of the endogenous gene (Napoli *et al.*, *Plant Cell* 2:279-289 (1990); van der Krol *et al.*, *Plant Cell* 2:291-299 (1990)). Cosuppression may result from stable transformation with a single copy nucleic acid molecule that is homologous to a nucleic acid sequence found with the cell (Proll and Meyer, *Plant J.* 2:465-475 (1992)) or with multiple copies of a nucleic acid molecule that is homologous to a nucleic acid sequence found with the cell (Mittlesten *et al.*, *Mol. Gen. Genet.* 244:325-330 (1994)). Genes, even though different, linked to homologous promoters may result in the cosuppression of the linked genes (Vaucheret, *C.R. Acad. Sci. III* 316:1471-1483 (1993); Flavell, *Proc. Natl. Acad. Sci. (U.S.A.)* 91:3490-3496 (1994)); van Blokland *et al.*, *Plant J.* 6:861-877 (1994); Jorgensen, *Trends Biotechnol.* 8:340-344 (1990); Meins and Kunz, In: *Gene Inactivation and Homologous Recombination in Plants*, Paszkowski (ed.), pp. 335-348, Kluwer Academic, Netherlands (1994)).

It is understood that one or more of the nucleic acids of the invention may be introduced into a plant cell and transcribed using an appropriate promoter with such transcription resulting in the cosuppression of an endogenous protein.

Antisense approaches are a way of preventing or reducing gene function by targeting the genetic material (Mol *et al.*, *FEBS Lett.* 268:427-430 (1990)). The objective of the antisense approach is to use a sequence complementary to the target gene to block its expression and create a mutant cell line or organism in which the level of a single chosen protein is selectively reduced or abolished. Antisense techniques have several advantages over other 'reverse genetic' approaches. The site of inactivation and its developmental effect can be manipulated by the choice of promoter for antisense genes or by the timing of external application or microinjection. Antisense can manipulate its specificity by selecting either unique regions of the target gene or regions where it shares homology to other related genes (Hiatt *et al.*, In: *Genetic Engineering*, Setlow (ed.), Vol. 11, New York: Plenum 49-63 (1989)).

Antisense RNA techniques involve introduction of RNA that is complementary to the target mRNA into cells, which results in specific RNA:RNA duplexes being formed by base pairing between the antisense substrate and the target mRNA (Green *et al.*, *Annu. Rev. Biochem.* 55:569-597 (1986)). Under one embodiment, the process involves the introduction and expression of an antisense gene sequence. Such a sequence is one in which part or all of the normal gene sequences are placed under a promoter in inverted

orientation so that the 'wrong' or complementary strand is transcribed into a noncoding antisense RNA that hybridizes with the target mRNA and interferes with its expression (Takayama and Inouye, *Crit. Rev. Biochem. Mol. Biol.* 25:155-184 (1990)). An antisense vector is constructed by standard procedures and introduced into cells by transformation, transfection, electroporation, microinjection, infection, *etc.* The type of transformation and choice of vector will determine whether expression is transient or stable. The promoter used for the antisense gene may influence the level, timing, tissue, specificity, or inducibility of the antisense inhibition.

It is understood that the activity of a protein in a plant cell may be reduced or depressed by growing a transformed plant cell containing a nucleic acid molecule whose non-transcribed strand encodes a protein or fragment thereof. A preferred protein whose activity can be reduced or depressed, by any method, is tMT2. In such an embodiment of the invention, it is preferred that the concentration of δ -tocopherol or δ -tocotrienol is increased. Another preferred protein whose activity can be reduced or depressed, by any method, is homogentisic acid dioxygenase.

Posttranscriptional gene silencing (PTGS) can result in virus immunity or gene silencing in plants. PTGS is induced by dsRNA and is mediated by an RNA-dependent RNA polymerase, present in the cytoplasm, which requires a dsRNA template. The dsRNA is formed by hybridization of complementary transgene mRNAs or complementary regions of the same transcript. Duplex formation can be accomplished by using transcripts from one sense gene and one antisense gene colocated in the plant genome, a single transcript that has self-complementarity, or sense and antisense transcripts from genes brought together by crossing. The dsRNA-dependent RNA polymerase makes a complementary strand from the transgene mRNA and RNase molecules attach to this complementary strand (cRNA). These cRNA-RNase molecules hybridize to the endogene mRNA and cleave the single-stranded RNA adjacent to the hybrid. The cleaved single-stranded RNAs are further degraded by other host RNases because one will lack a capped 5' end and the other will lack a poly(A) tail (Waterhouse *et al.*, *PNAS* 95:13959-13964 (1998)).

It is understood that one or more of the nucleic acids of the invention may be introduced into a plant cell and transcribed using an appropriate promoter with such transcription resulting in the postranscriptional gene silencing of an endogenous transcript.

Antibodies have been expressed in plants (Hiatt *et al.*, *Nature* 342:76-78 (1989); Conrad and Fielder, *Plant Mol. Biol.* 26:1023-1030 (1994)). Cytoplasmic expression of a scFv (single-chain Fv antibody) has been reported to delay infection by artichoke mottled crinkle virus. Transgenic plants that express antibodies directed against endogenous proteins may exhibit a physiological effect (Philips *et al.*, *EMBO J.* 16:4489-4496 (1997); Marion-Poll, *Trends in Plant Science* 2:447-448 (1997)). For example, expressed anti-abscisic antibodies have been reported to result in a general perturbation of seed development (Philips *et al.*, *EMBO J.* 16:4489-4496 (1997)).

Antibodies that are catalytic may also be expressed in plants (abzymes). The principle behind abzymes is that since antibodies may be raised against many molecules, this recognition ability can be directed toward generating antibodies that bind transition states to force a chemical reaction forward (Persidas, *Nature Biotechnology* 15:1313-1315 (1997); Baca *et al.*, *Ann. Rev. Biophys. Biomol. Struct.* 26:461-493 (1997)). The catalytic abilities of abzymes may be enhanced by site directed mutagenesis. Examples of abzymes are, for example, set forth in U.S. Patent: 5,658,753; U.S. Patent 5,632,990; U.S. Patent 5,631,137; U.S. Patent 5,602,015; U.S. Patent 5,559,538; U.S. Patent 5,576,174; U.S. Patent 5,500,358; U.S. Patent 5,318,897; U.S. Patent 5,298,409; U.S. Patent 5,258,289 and U.S. Patent 5,194,585.

It is understood that any of the antibodies of the invention may be expressed in plants and that such expression can result in a physiological effect. It is also understood that any of the expressed antibodies may be catalytic.

The present invention also provides for parts of the plants, particularly reproductive or storage parts, of the present invention. Plant parts, without limitation, include seed, endosperm, ovule and pollen. In a particularly preferred embodiment of the present invention, the plant part is a seed. In one embodiment the seed is a constituent of animal feed.

In another embodiment, the plant part is a fruit, more preferably a fruit with enhanced shelf life. In another preferred embodiment, the fruit has increased levels of a tocopherol. In another preferred embodiment, the fruit has increased levels of a tocotrienol.

The present invention also provides a container of over about 10,000, more preferably about 20,000, and even more preferably about 40,000 seeds where over about

10%, more preferably about 25%, more preferably about 50% and even more preferably about 75% or 90% of the seeds are seeds derived from a plant of the present invention.

The present invention also provides a container of over about 10 kg, more preferably about 25 kg, and even more preferably about 50 kg seeds where over about
5 10%, more preferably about 25%, more preferably about 50% and even more preferably about 75% or 90% of the seeds are seeds derived from a plant of the present invention.

Any of the plants or parts thereof of the present invention may be processed to produce a feed, meal, protein, or oil preparation, including oil preparations high in total tocopherol content and oil preparations high in any one or more of each tocopherol
10 component listed herein. A particularly preferred plant part for this purpose is a seed. In a preferred embodiment the feed, meal, protein or oil preparation is designed for livestock animals or humans, or both. Methods to produce feed, meal, protein and oil preparations are known in the art. See, for example, U.S. Patents 4,957,748, 5,100,679, 5,219,596, 5,936,069, 6,005,076, 6,146,669, and 6,156,227. In a preferred embodiment, the protein
15 preparation is a high protein preparation. Such a high protein preparation preferably has a protein content of greater than about 5% w/v, more preferably about 10% w/v, and even more preferably about 15% w/v. In a preferred oil preparation, the oil preparation is a high oil preparation with an oil content derived from a plant or part thereof of the present invention of greater than about 5% w/v, more preferably about 10% w/v, and even more
20 preferably about 15% w/v. In a preferred embodiment the oil preparation is a liquid and of a volume greater than about 1, 5, 10 or 50 liters. The present invention provides for oil produced from plants of the present invention or generated by a method of the present invention. Such an oil may exhibit enhanced oxidative stability. Also, such oil may be a minor or major component of any resultant product. Moreover, such oil may be blended
25 with other oils. In a preferred embodiment, the oil produced from plants of the present invention or generated by a method of the present invention constitutes greater than about 0.5%, 1%, 5%, 10%, 25%, 50%, 75% or 90% by volume or weight of the oil component of any product. In another embodiment, the oil preparation may be blended and can constitute greater than about 10%, 25%, 35%, 50% or 75% of the blend by volume. Oil produced
30 from a plant of the present invention can be admixed with one or more organic solvents or petroleum distillates.

Plants of the present invention can be part of or generated from a breeding program. The choice of breeding method depends on the mode of plant reproduction, the heritability

of the trait(s) being improved, and the type of cultivar used commercially (e.g., F₁ hybrid cultivar, pureline cultivar, etc). Selected, non-limiting approaches, for breeding the plants of the present invention are set forth below. A breeding program can be enhanced using marker assisted selection of the progeny of any cross. It is further understood that any commercial and non-commercial cultivars can be utilized in a breeding program. Factors such as, for example, emergence vigor, vegetative vigor, stress tolerance, disease resistance, branching, flowering, seed set, seed size, seed density, standability, and threshability etc. will generally dictate the choice.

For highly heritable traits, a choice of superior individual plants evaluated at a single location will be effective, whereas for traits with low heritability, selection should be based on mean values obtained from replicated evaluations of families of related plants. Popular selection methods commonly include pedigree selection, modified pedigree selection, mass selection, and recurrent selection. In a preferred embodiment a backcross or recurrent breeding program is undertaken.

The complexity of inheritance influences choice of the breeding method. Backcross breeding can be used to transfer one or a few favorable genes for a highly heritable trait into a desirable cultivar. This approach has been used extensively for breeding disease-resistant cultivars. Various recurrent selection techniques are used to improve quantitatively inherited traits controlled by numerous genes. The use of recurrent selection in self-pollinating crops depends on the ease of pollination, the frequency of successful hybrids from each pollination, and the number of hybrid offspring from each successful cross.

Breeding lines can be tested and compared to appropriate standards in environments representative of the commercial target area(s) for two or more generations. The best lines are candidates for new commercial cultivars; those still deficient in traits may be used as parents to produce new populations for further selection.

One method of identifying a superior plant is to observe its performance relative to other experimental plants and to a widely grown standard cultivar. If a single observation is inconclusive, replicated observations can provide a better estimate of its genetic worth. A breeder can select and cross two or more parental lines, followed by repeated selfing and selection, producing many new genetic combinations.

The development of new cultivars requires the development and selection of varieties, the crossing of these varieties and the selection of superior hybrid crosses. The hybrid seed can be produced by manual crosses between selected male-fertile parents or by using male sterility systems. Hybrids are selected for certain single gene traits such as pod color, flower color, seed yield, pubescence color, or herbicide resistance, which indicate that the seed is truly a hybrid. Additional data on parental lines, as well as the phenotype of the hybrid, influence the breeder's decision whether to continue with the specific hybrid cross.

Pedigree breeding and recurrent selection breeding methods can be used to develop cultivars from breeding populations. Breeding programs combine desirable traits from two or more cultivars or various broad-based sources into breeding pools from which cultivars are developed by selfing and selection of desired phenotypes. New cultivars can be evaluated to determine which have commercial potential.

Pedigree breeding is used commonly for the improvement of self-pollinating crops. Two parents who possess favorable, complementary traits are crossed to produce an F_1 . A F_2 population is produced by selfing one or several F_1 's. Selection of the best individuals from the best families is carried out. Replicated testing of families can begin in the F_4 generation to improve the effectiveness of selection for traits with low heritability. At an advanced stage of inbreeding (*i.e.*, F_6 and F_7), the best lines or mixtures of phenotypically similar lines are tested for potential release as new cultivars.

Backcross breeding has been used to transfer genes for a simply inherited, highly heritable trait into a desirable homozygous cultivar or inbred line, which is the recurrent parent. The source of the trait to be transferred is called the donor parent. The resulting plant is expected to have the attributes of the recurrent parent (*e.g.*, cultivar) and the desirable trait transferred from the donor parent. After the initial cross, individuals possessing the phenotype of the donor parent are selected and repeatedly crossed (backcrossed) to the recurrent parent. The resulting parent is expected to have the attributes of the recurrent parent (*e.g.*, cultivar) and the desirable trait transferred from the donor parent.

The single-seed descent procedure in the strict sense refers to planting a segregating population, harvesting a sample of one seed per plant, and using the one-seed sample to plant the next generation. When the population has been advanced from the F_2 to the

desired level of inbreeding, the plants from which lines are derived will each trace to different F_2 individuals. The number of plants in a population declines each generation due to failure of some seeds to germinate or some plants to produce at least one seed. As a result, not all of the F_2 plants originally sampled in the population will be represented by a progeny when generation advance is completed.

In a multiple-seed procedure, breeders commonly harvest one or more pods from each plant in a population and thresh them together to form a bulk. Part of the bulk is used to plant the next generation and part is put in reserve. The procedure has been referred to as modified single-seed descent or the pod-bulk technique.

The multiple-seed procedure has been used to save labor at harvest. It is considerably faster to thresh pods with a machine than to remove one seed from each by hand for the single-seed procedure. The multiple-seed procedure also makes it possible to plant the same number of seed of a population each generation of inbreeding.

Descriptions of other breeding methods that are commonly used for different traits and crops can be found in one of several reference books (*e.g.* Fehr, *Principles of Cultivar Development* Vol. 1, pp. 2-3 (1987))).

A transgenic plant of the present invention may also be reproduced using apomixis. Apomixis is a genetically controlled method of reproduction in plants where the embryo is formed without union of an egg and a sperm. There are three basic types of apomictic reproduction: 1) apospory where the embryo develops from a chromosomally unreduced egg in an embryo sac derived from the nucleus, 2) diplospory where the embryo develops from an unreduced egg in an embryo sac derived from the megaspore mother cell, and 3) adventitious embryo where the embryo develops directly from a somatic cell. In most forms of apomixis, pseudogamy or fertilization of the polar nuclei to produce endosperm is necessary for seed viability. In apospory, a nurse cultivar can be used as a pollen source for endosperm formation in seeds. The nurse cultivar does not affect the genetics of the aposporous apomictic cultivar since the unreduced egg of the cultivar develops parthenogenetically, but makes possible endosperm production. Apomixis is economically important, especially in transgenic plants, because it causes any genotype, no matter how heterozygous, to breed true. Thus, with apomictic reproduction, heterozygous transgenic plants can maintain their genetic fidelity throughout repeated life cycles. Methods for the production of apomictic plants are known in the art. *See*, U.S. Patent 5,811,636.

OTHER ORGANISMS

A nucleic acid of the present invention may be introduced into any cell or organism such as a mammalian cell, mammal, fish cell, fish, bird cell, bird, algae cell, algae, fungal cell, fungi, or bacterial cell. A protein of the present invention may be produced in an appropriate cell or organism. Preferred host and transformants include: fungal cells such as *Aspergillus*, yeasts, mammals, particularly bovine and porcine, insects, bacteria, and algae. Particularly preferred bacteria are *Agrobacterium tumefaciens* and *E. coli*.

Methods to transform such cells or organisms are known in the art (EP 0 238 023; Yelton *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)*, 81:1470-1474 (1984); Malardier *et al.*, *Gene*, 78:147-156 (1989); Becker and Guarente, In: Abelson and Simon (eds.), *Guide to Yeast Genetics and Molecular Biology, Method Enzymol.*, Vol. 194, pp. 182-187, Academic Press, Inc., New York; Ito *et al.*, *J. Bacteriology*, 153:163 (1983) Hinnen *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)*, 75:1920 (1978); Bennett and LaSure (eds.), *More Gene Manipulation in fungi*, Academic Press, CA (1991)). Methods to produce proteins of the present invention are also known (Kudla *et al.*, *EMBO*, 9:1355-1364 (1990); Jarai and Buxton, *Current Genetics*, 26:2238-2244 (1994); Verdier, *Yeast*, 6:271-297 (1990); MacKenzie *et al.*, *Journal of Gen. Microbiol.*, 139:2295-2307 (1993); Hartl *et al.*, *TIBS*, 19:20-25 (1994); Bergenron *et al.*, *TIBS*, 19:124-128 (1994); Demolder *et al.*, *J. Biotechnology*, 32:179-189 (1994); Craig, *Science*, 260:1902-1903 (1993); Gething and Sambrook, *Nature*, 355:33-45 (1992); Puig and Gilbert, *J. Biol. Chem.*, 269:7764-7771 (1994); Wang and Tsou, *FASEB Journal*, 7:1515-1517 (1993); Robinson *et al.*, *Bio/Technology*, 1:381-384 (1994); Enderlin and Ogrydziak, *Yeast*, 10:67-79 (1994); Fuller *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)*, 86:1434-1438 (1989); Julius *et al.*, *Cell*, 37:1075-1089 (1984); Julius *et al.*, *Cell* 32:839-852 (1983).

In a preferred embodiment, overexpression of a protein or fragment thereof of the present invention in a cell or organism provides in that cell or organism, relative to an untransformed cell or organism with a similar genetic background, an increased level of tocopherols.

In a preferred embodiment, overexpression of a protein or fragment thereof of the present invention in a cell or organism provides in that cell or organism, relative to an untransformed cell or organism with a similar genetic background, an increased level of α -tocopherols.

In a preferred embodiment, overexpression of a protein or fragment thereof of the present invention in a cell or organism provides in that cell or organism, relative to an untransformed cell or organism with a similar genetic background, an increased level of γ -tocopherols.

5 In another preferred embodiment, overexpression of a protein or fragment thereof of the present invention in a cell or organism provides in that cell or organism, relative to an untransformed cell or organism with a similar genetic background, an increased level of α -tocotrienols.

10 In another preferred embodiment, overexpression of a protein or fragment thereof of the present invention in a cell or organism provides in that cell or organism, relative to an untransformed cell or organism with a similar genetic background, an increased level of γ -tocotrienols.

ANTIBODIES

One aspect of the invention concerns antibodies, single-chain antigen binding
15 molecules, or other proteins that specifically bind to one or more of the protein or peptide molecules of the invention and their homologs, fusions or fragments. In a particularly preferred embodiment, the antibody specifically binds to a protein having the amino acid sequence set forth in SEQ ID NOs: 16 through 38 or fragments thereof. In another
20 embodiment, the antibody specifically binds to a fusion protein comprising an amino acid sequence selected from the amino acid sequence set forth in SEQ ID NOs: 16 through 38 or fragments thereof. Antibodies of the invention may be used to quantitatively or qualitatively detect the protein or peptide molecules of the invention, or to detect post translational modifications of the proteins. As used herein, an antibody or peptide is said to
25 “specifically bind” to a protein or peptide molecule of the invention if such binding is not competitively inhibited by the presence of non-related molecules.

Nucleic acid molecules that encode all or part of the protein of the invention can be expressed, via recombinant means, to yield protein or peptides that can in turn be used to elicit antibodies that are capable of binding the expressed protein or peptide. Such
30 antibodies may be used in immunoassays for that protein. Such protein-encoding molecules, or their fragments may be a “fusion” molecule (*i.e.*, a part of a larger nucleic acid molecule) such that, upon expression, a fusion protein is produced. It is understood

that any of the nucleic acid molecules of the invention may be expressed, via recombinant means, to yield proteins or peptides encoded by these nucleic acid molecules.

The antibodies that specifically bind proteins and protein fragments of the invention may be polyclonal or monoclonal and may comprise intact immunoglobulins, or antigen
5 binding portions of immunoglobulins fragments (such as (F(ab'), F(ab')₂), or single-chain immunoglobulins producible, for example, via recombinant means. It is understood that practitioners are familiar with the standard resource materials that describe specific conditions and procedures for the construction, manipulation and isolation of antibodies (see, for example, Harlow and Lane, In: *Antibodies: A Laboratory Manual*, Cold Spring
10 Harbor Press, Cold Spring Harbor, New York (1988)).

As discussed below, such antibody molecules or their fragments may be used for diagnostic purposes. Where the antibodies are intended for diagnostic purposes, it may be desirable to derivatize them, for example with a ligand group (such as biotin) or a detectable marker group (such as a fluorescent group, a radioisotope or an enzyme).

15 The ability to produce antibodies that bind the protein or peptide molecules of the invention permits the identification of mimetic compounds derived from those molecules. These mimetic compounds may contain a fragment of the protein or peptide or merely a structurally similar region and nonetheless exhibits an ability to specifically bind to antibodies directed against that compound.

20 EXEMPLARY USES

Nucleic acid molecules and fragments thereof of the invention may be employed to obtain other nucleic acid molecules from the same species (nucleic acid molecules from corn may be utilized to obtain other nucleic acid molecules from corn). Such nucleic acid molecules include the nucleic acid molecules that encode the complete coding sequence of
25 a protein and promoters and flanking sequences of such molecules. In addition, such nucleic acid molecules include nucleic acid molecules that encode for other isozymes or gene family members. Such molecules can be readily obtained by using the above-described nucleic acid molecules or fragments thereof to screen cDNA or genomic libraries. Methods for forming such libraries are well known in the art.

30 Nucleic acid molecules and fragments thereof of the invention may also be employed to obtain nucleic acid homologs. Such homologs include the nucleic acid molecules of plants and other organisms, including bacteria and fungi, including the

nucleic acid molecules that encode, in whole or in part, protein homologues of other plant species or other organisms, sequences of genetic elements, such as promoters and transcriptional regulatory elements. Such molecules can be readily obtained by using the above-described nucleic acid molecules or fragments thereof to screen cDNA or genomic

5 libraries obtained from such plant species. Methods for forming such libraries are well known in the art. Such homolog molecules may differ in their nucleotide sequences from those found in one or more of SEQ ID NOs: 1 through 15, and complements thereof because complete complementarity is not needed for stable hybridization. The nucleic acid molecules of the invention therefore also include molecules that, although capable of
10 specifically hybridizing with the nucleic acid molecules may lack "complete complementarity."

Any of a variety of methods may be used to obtain one or more of the above-described nucleic acid molecules (Zamechik *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 83:4143-4146 (1986); Goodchild *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:5507-5511 (1988);
15 Wickstrom *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:1028-1032 (1988); Holt *et al.*, *Molec. Cell. Biol.* 8:963-973 (1988); Gerwitz *et al.*, *Science* 242:1303-1306 (1988); Anfossi *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:3379-3383 (1989); Becker *et al.*, *EMBO J.* 8:3685-3691 (1989)). Automated nucleic acid synthesizers may be employed for this purpose. In lieu of such synthesis, the disclosed nucleic acid molecules may be used to define a pair of primers
20 that can be used with the polymerase chain reaction (Mullis *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* 51:263-273 (1986); Erlich *et al.*, European Patent 50,424; European Patent 84,796; European Patent 258,017; European Patent 237,362; Mullis, European Patent 201,184; Mullis *et al.*, U.S. Patent 4,683,202; Erlich, U.S. Patent 4,582,788; and Saiki *et al.*, U.S. Patent 4,683,194) to amplify and obtain any desired nucleic acid molecule
25 or fragment.

Promoter sequences and other genetic elements, including but not limited to transcriptional regulatory flanking sequences, associated with one or more of the disclosed nucleic acid sequences can also be obtained using the disclosed nucleic acid sequence provided herein. In one embodiment, such sequences are obtained by incubating nucleic
30 acid molecules of the present invention with members of genomic libraries and recovering clones that hybridize to such nucleic acid molecules thereof. In a second embodiment, methods of "chromosome walking," or inverse PCR may be used to obtain such sequences (Frohman *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:8998-9002 (1988); Ohara *et al.*, *Proc.*

Natl. Acad. Sci. (U.S.A.) 86:5673-5677 (1989); Pang *et al.*, *Biotechniques* 22:1046-1048 (1977); Huang *et al.*, *Methods Mol. Biol.* 69:89-96 (1997); Huang *et al.*, *Method Mol. Biol.* 67:287-294 (1997); Benkel *et al.*, *Genet. Anal.* 13:123-127 (1996); Hartl *et al.*, *Methods Mol. Biol.* 58:293-301 (1996)). The term "chromosome walking" means a process of
5 extending a genetic map by successive hybridization steps.

The nucleic acid molecules of the invention may be used to isolate promoters of cell enhanced, cell specific, tissue enhanced, tissue specific, developmentally or environmentally regulated expression profiles. Isolation and functional analysis of the 5' flanking promoter sequences of these genes from genomic libraries, for example, using
10 genomic screening methods and PCR techniques would result in the isolation of useful promoters and transcriptional regulatory elements. These methods are known to those of skill in the art and have been described (See, for example, Birren *et al.*, *Genome Analysis: Analyzing DNA*, 1, (1997), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). Promoters obtained utilizing the nucleic acid molecules of the invention could also
15 be modified to affect their control characteristics. Examples of such modifications would include but are not limited to enhancer sequences. Such genetic elements could be used to enhance gene expression of new and existing traits for crop improvement.

Another subset of the nucleic acid molecules of the invention includes nucleic acid molecules that are markers. The markers can be used in a number of conventional ways in
20 the field of molecular genetics. Such markers include nucleic acid molecules SEQ ID NOs: 1 through 15, complements thereof, and fragments of either that can act as markers and other nucleic acid molecules of the present invention that can act as markers.

Genetic markers of the invention include "dominant" or "codominant" markers. "Codominant markers" reveal the presence of two or more alleles (two per diploid
25 individual) at a locus. "Dominant markers" reveal the presence of only a single allele per locus. The presence of the dominant marker phenotype (e.g., a band of DNA) is an indication that one allele is in either the homozygous or heterozygous condition. The absence of the dominant marker phenotype (e.g., absence of a DNA band) is merely evidence that "some other" undefined allele is present. In the case of populations where
30 individuals are predominantly homozygous and loci are predominately dimorphic, dominant and codominant markers can be equally valuable. As populations become more heterozygous and multi-allelic, codominant markers often become more informative of the

genotype than dominant markers. Marker molecules can be, for example, capable of detecting polymorphisms such as single nucleotide polymorphisms (SNPs).

The genomes of animals and plants naturally undergo spontaneous mutation in the course of their continuing evolution (Gusella, *Ann. Rev. Biochem.* 55:831-854 (1986)). A
5 "polymorphism" is a variation or difference in the sequence of the gene or its flanking regions that arises in some of the members of a species. The variant sequence and the "original" sequence co-exist in the species' population. In some instances, such co-existence is in stable or quasi-stable equilibrium.

A polymorphism is thus said to be "allelic," in that, due to the existence of the
10 polymorphism, some members of a population may have the original sequence (i.e., the original "allele") whereas other members may have the variant sequence (i.e., the variant "allele"). In the simplest case, only one variant sequence may exist and the polymorphism is thus said to be di-allelic. In other cases, the species' population may contain multiple alleles and the polymorphism is termed tri-allelic, etc. A single gene may have multiple
15 different unrelated polymorphisms. For example, it may have a di-allelic polymorphism at one site and a multi-allelic polymorphism at another site.

The variation that defines the polymorphism may range from a single nucleotide variation to the insertion or deletion of extended regions within a gene. In some cases, the DNA sequence variations are in regions of the genome that are characterized by short
20 tandem repeats (STRs) that include tandem di- or tri-nucleotide repeated motifs of nucleotides. Polymorphisms characterized by such tandem repeats are referred to as "variable number tandem repeat" ("VNTR") polymorphisms. VNTRs have been used in identity analysis (Weber, U.S. Patent 5,075,217; Armour *et al.*, *FEBS Lett.* 307:113-115 (1992); Jones *et al.*, *Eur. J. Haematol.* 39:144-147 (1987); Horn *et al.*, PCT Patent
25 Application WO91/14003; Jeffreys, European Patent Application 370,719; Jeffreys, U.S. Patent 5,175,082; Jeffreys *et al.*, *Amer. J. Hum. Genet.* 39:11-24 (1986); Jeffreys *et al.*, *Nature* 316:76-79 (1985); Gray *et al.*, *Proc. R. Acad. Soc. Lond.* 243:241-253 (1991); Moore *et al.*, *Genomics* 10:654-660 (1991); Jeffreys *et al.*, *Anim. Genet.* 18:1-15 (1987); Hillel *et al.*, *Anim. Genet.* 20:145-155 (1989); Hillel *et al.*, *Genet.* 124:783-789 (1990)).

30 The detection of polymorphic sites in a sample of DNA may be facilitated through the use of nucleic acid amplification methods. Such methods specifically increase the concentration of polynucleotides that span the polymorphic site, or include that site and

sequences located either distal or proximal to it. Such amplified molecules can be readily detected by gel electrophoresis or other means.

In an alternative embodiment, such polymorphisms can be detected through the use of a marker nucleic acid molecule that is physically linked to such polymorphism(s). For this purpose, marker nucleic acid molecules comprising a nucleotide sequence of a polynucleotide located within 1 mb of the polymorphism(s) and more preferably within 100kb of the polymorphism(s) and most preferably within 10kb of the polymorphism(s) can be employed.

The identification of a polymorphism can be determined in a variety of ways. By correlating the presence or absence of it in a plant with the presence or absence of a phenotype, it is possible to predict the phenotype of that plant. If a polymorphism creates or destroys a restriction endonuclease cleavage site, or if it results in the loss or insertion of DNA (e.g., a VNTR polymorphism), it will alter the size or profile of the DNA fragments that are generated by digestion with that restriction endonuclease. As such, organisms that possess a variant sequence can be distinguished from those having the original sequence by restriction fragment analysis. Polymorphisms that can be identified in this manner are termed "restriction fragment length polymorphisms" ("RFLPs") (Glassberg, UK Patent Application 2135774; Skolnick *et al.*, *Cytogen. Cell Genet.* 32:58-67 (1982); Botstein *et al.*, *Ann. J. Hum. Genet.* 32:314-331 (1980); Fischer *et al.*, (PCT Application WO90/13668; Uhlen, PCT Application WO90/11369).

Polymorphisms can also be identified by Single Strand Conformation Polymorphism (SSCP) analysis (Elles, *Methods in Molecular Medicine: Molecular Diagnosis of Genetic Diseases*, Humana Press (1996)); Orita *et al.*, *Genomics* 5:874-879 (1989)). A number of protocols have been described for SSCP including, but not limited to, Lee *et al.*, *Anal. Biochem.* 205:289-293 (1992); Suzuki *et al.*, *Anal. Biochem.* 192:82-84 (1991); Lo *et al.*, *Nucleic Acids Research* 20:1005-1009 (1992); Sarkar *et al.*, *Genomics* 13:441-443 (1992). It is understood that one or more of the nucleic acids of the invention, may be utilized as markers or probes to detect polymorphisms by SSCP analysis.

Polymorphisms may also be found using a DNA fingerprinting technique called amplified fragment length polymorphism (AFLP), which is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA to profile that DNA (Vos *et al.*, *Nucleic Acids Res.* 23:4407-4414 (1995)). This method allows for the

specific co-amplification of high numbers of restriction fragments, which can be visualized by PCR without knowledge of the nucleic acid sequence. It is understood that one or more of the nucleic acids of the invention may be utilized as markers or probes to detect polymorphisms by AFLP analysis or for fingerprinting RNA.

5 Polymorphisms may also be found using random amplified polymorphic DNA (RAPD) (Williams *et al.*, *Nucl. Acids Res.* 18:6531-6535 (1990)) and cleaveable amplified polymorphic sequences (CAPS) (Lyamichev *et al.*, *Science* 260:778-783 (1993)). It is understood that one or more of the nucleic acid molecules of the invention, may be utilized as markers or probes to detect polymorphisms by RAPD or CAPS analysis.

10 Single Nucleotide Polymorphisms (SNPs) generally occur at greater frequency than other polymorphic markers and are spaced with a greater uniformity throughout a genome than other reported forms of polymorphism. The greater frequency and uniformity of SNPs means that there is greater probability that such a polymorphism will be found near or in a genetic locus of interest than would be the case for other polymorphisms. SNPs are located
15 in protein-coding regions and noncoding regions of a genome. Some of these SNPs may result in defective or variant protein expression (e.g., as a result of mutations or defective splicing). Analysis (genotyping) of characterized SNPs can require only a plus/minus assay rather than a lengthy measurement, permitting easier automation.

SNPs can be characterized using any of a variety of methods. Such methods
20 include the direct or indirect sequencing of the site, the use of restriction enzymes (Botstein *et al.*, *Am. J. Hum. Genet.* 32:314-331 (1980); Konieczny and Ausubel, *Plant J.* 4:403-410 (1993)), enzymatic and chemical mismatch assays (Myers *et al.*, *Nature* 313:495-498 (1985)), allele-specific PCR (Newton *et al.*, *Nucl. Acids Res.* 17:2503-2516 (1989); Wu *et al.*, *Proc. Natl. Acad. Sci. USA* 86:2757-2760 (1989)), ligase chain reaction (Barany, *Proc.*
25 *Natl. Acad. Sci. USA* 88:189-193 (1991)), single-strand conformation polymorphism analysis (Labruno *et al.*, *Am. J. Hum. Genet.* 48:1115-1120 (1991)), single base primer extension (Kuppuswamy *et al.*, *Proc. Natl. Acad. Sci. USA* 88:1143-1147 (1991)), Goelet
US 6,004,744; Goelet 5,888,819), solid-phase ELISA-based oligonucleotide ligation assays (Nikiforov *et al.*, *Nucl. Acids Res.* 22:4167-4175 (1994)), dideoxy fingerprinting (Sarkar *et al.*, *Genomics* 13:441-443 (1992)), oligonucleotide fluorescence-quenching assays (Livak
30 *et al.*, *PCR Methods Appl.* 4:357-362 (1995a)), 5'-nuclease allele-specific hybridization TaqMan™ assay (Livak *et al.*, *Nature Genet.* 9:341-342 (1995)), template-directed dye-terminator incorporation (TDI) assay (Chen and Kwok, *Nucl. Acids Res.* 25:347-353

(1997)), allele-specific molecular beacon assay (Tyagi *et al.*, *Nature Biotech.* 16:49-53 (1998)), PinPoint assay (Haff and Smirnov, *Genome Res.* 7:378-388 (1997)), dCAPS analysis (Neff *et al.*, *Plant J.* 14:387-392 (1998)), pyrosequencing (Ronaghi *et al.*, *Analytical Biochemistry* 267:65-71 (1999); Ronaghi *et al.* PCT application WO 98/13523; Nyren *et al.* PCT application WO 98/28440; www.pyrosequencing.com), using mass spectrometry, *e.g.* the Masscode™ system (Howbert *et al.* PCT application, WO 99/05319; Howbert *et al.* PCT application WO 97/27331; www.rapigene.com; Becker *et al.* PCT application WO 98/26095; Becker *et al.* PCT application; WO 98/12355; Becker *et al.* PCT application WO 97/33000; Monforte *et al.* US 5,965,363), invasive cleavage of oligonucleotide probes (Lyamichev *et al.* *Nature Biotechnology* 17:292-296; www.twt.com), and using high density oligonucleotide arrays (Hacia *et al.* *Nature Genetics* 22:164-167; www.affymetrix.com).

Polymorphisms may also be detected using allele-specific oligonucleotides (ASO), which, can be for example, used in combination with hybridization based technology including Southern, Northern, and dot blot hybridizations, reverse dot blot hybridizations and hybridizations performed on microarray and related technology.

The stringency of hybridization for polymorphism detection is highly dependent upon a variety of factors, including length of the allele-specific oligonucleotide, sequence composition, degree of complementarity (*i.e.* presence or absence of base mismatches), concentration of salts and other factors such as formamide, and temperature. These factors are important both during the hybridization itself and during subsequent washes performed to remove target polynucleotide that is not specifically hybridized. In practice, the conditions of the final, most stringent wash are most critical. In addition, the amount of target polynucleotide that is able to hybridize to the allele-specific oligonucleotide is also governed by such factors as the concentration of both the ASO and the target polynucleotide, the presence and concentration of factors that act to "tie up" water molecules, so as to effectively concentrate the reagents (*e.g.*, PEG, dextran, dextran sulfate, *etc.*), whether the nucleic acids are immobilized or in solution, and the duration of hybridization and washing steps.

Hybridizations are preferably performed below the melting temperature (T_m) of the ASO. The closer the hybridization and/or washing step is to the T_m , the higher the stringency. T_m for an oligonucleotide may be approximated, for example, according to the following formula: $T_m = 81.5 + 16.6 \times (\log_{10}[\text{Na}^+]) + 0.41 \times (\%G+C) - 675/n$; where

[Na⁺] is the molar salt concentration of Na⁺ or any other suitable cation and n = number of bases in the oligonucleotide. Other formulas for approximating T_m are available and are known to those of ordinary skill in the art.

Stringency is preferably adjusted so as to allow a given ASO to differentially
5 hybridize to a target polynucleotide of the correct allele and a target polynucleotide of the incorrect allele. Preferably, there will be at least a two-fold differential between the signal produced by the ASO hybridizing to a target polynucleotide of the correct allele and the level of the signal produced by the ASO cross-hybridizing to a target polynucleotide of the incorrect allele (e.g., an ASO specific for a mutant allele cross-hybridizing to a wild-type
10 allele). In more preferred embodiments of the present invention, there is at least a five-fold signal differential. In highly preferred embodiments of the present invention, there is at least an order of magnitude signal differential between the ASO hybridizing to a target polynucleotide of the correct allele and the level of the signal produced by the ASO cross-hybridizing to a target polynucleotide of the incorrect allele.

15 While certain methods for detecting polymorphisms are described herein, other detection methodologies may be utilized. For example, additional methodologies are known and set forth, in Birren *et al.*, *Genome Analysis*, 4:135-186, *A Laboratory Manual. Mapping Genomes*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1999); Maliga *et al.*, *Methods in Plant Molecular Biology. A Laboratory Course Manual*, Cold
20 Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1995); Paterson, *Biotechnology Intelligence Unit: Genome Mapping in Plants*, R.G. Landes Co., Georgetown, TX, and Academic Press, San Diego, CA (1996); *The Corn Handbook*, Freeling and Walbot, eds., Springer-Verlag, New York, NY (1994); *Methods in Molecular Medicine: Molecular Diagnosis of Genetic Diseases*, Elles, ed., Humana Press, Totowa, NJ (1996); Clark, ed.,
25 *Plant Molecular Biology: A Laboratory Manual*, Clark, ed., Springer-Verlag, Berlin, Germany (1997).

Factors for marker-assisted selection in a plant breeding program are: (1) the marker(s) should co-segregate or be closely linked with the desired trait; (2) an efficient means of screening large populations for the molecular marker(s) should be available; and
30 (3) the screening technique should have high reproducibility across laboratories and preferably be economical to use and be user-friendly.

The genetic linkage of marker molecules can be established by a gene mapping model such as, without limitation, the flanking marker model reported by Lander and Botstein, *Genetics* 121:185-199 (1989) and the interval mapping, based on maximum likelihood methods described by Lander and Botstein, *Genetics* 121:185-199 (1989) and implemented in the software package MAPMAKER/QTL (Lincoln and Lander, *Mapping Genes Controlling Quantitative Traits Using MAPMAKER/QTL*, Whitehead Institute for Biomedical Research, Massachusetts, (1990). Additional software includes Qgene, Version 2.23 (1996), Department of Plant Breeding and Biometry, 266 Emerson Hall, Cornell University, Ithaca, NY). Use of Qgene software is a particularly preferred approach.

A maximum likelihood estimate (MLE) for the presence of a marker is calculated, together with an MLE assuming no QTL effect, to avoid false positives. A \log_{10} of an odds ratio (LOD) is then calculated as: $\text{LOD} = \log_{10}(\text{MLE for the presence of a QTL} / \text{MLE given no linked QTL})$.

The LOD score essentially indicates how much more likely the data are to have arisen assuming the presence of a QTL than in its absence. The LOD threshold value for avoiding a false positive with a given confidence, say 95%, depends on the number of markers and the length of the genome. Graphs indicating LOD thresholds are set forth in Lander and Botstein, *Genetics* 121:185-199 (1989) and further described by Arús and Moreno-González, *Plant Breeding*, Hayward *et al.*, (eds.) Chapman & Hall, London, pp. 314-331 (1993).

In a preferred embodiment of the present invention the nucleic acid marker exhibits a LOD score of greater than 2.0, more preferably 2.5, even more preferably greater than 3.0 or 4.0 with the trait or phenotype of interest. In a preferred embodiment, the trait of interest is altered tocopherol levels or compositions or altered tocotrienol levels or compositions.

Additional models can be used. Many modifications and alternative approaches to interval mapping have been reported, including the use of non-parametric methods (Kruglyak and Lander, *Genetics* 139:1421-1428 (1995)). Multiple regression methods or models can also be used, in which the trait is regressed on a large number of markers (Jansen, *Biometrics in Plant Breeding*, van Oijen and Jansen (eds.), Proceedings of the Ninth Meeting of the Eucarpia Section Biometrics in Plant Breeding, The Netherlands, pp.

116-124 (1994); Weber and Wricke, *Advances in Plant Breeding*, Blackwell, Berlin, 16 (1994)). Procedures combining interval mapping with regression analysis, whereby the phenotype is regressed onto a single putative QTL at a given marker interval and at the same time onto a number of markers that serve as 'cofactors,' have been reported by Jansen and Stam, *Genetics* 136:1447-1455 (1994), and Zeng, *Genetics* 136:1457-1468 (1994).
5 Generally, the use of cofactors reduces the bias and sampling error of the estimated QTL positions (Utz and Melchinger, *Biometrics in Plant Breeding*, van Oijen and Jansen (eds.) Proceedings of the Ninth Meeting of the Eucarpia Section Biometrics in Plant Breeding, The Netherlands, pp. 195-204 (1994), thereby improving the precision and efficiency of
10 QTL mapping (Zeng, *Genetics* 136:1457-1468 (1994)). These models can be extended to multi-environment experiments to analyze genotype-environment interactions (Jansen *et al.*, *Theo. Appl. Genet.* 91:33-37 (1995)).

It is understood that one or more of the nucleic acid molecules of the invention may be used as molecular markers. It is also understood that one or more of the protein
15 molecules of the invention may be used as molecular markers.

In a preferred embodiment, the polymorphism is present and screened for in a mapping population, *e.g.* a collection of plants capable of being used with markers such as polymorphic markers to map genetic position of traits. The choice of appropriate mapping population often depends on the type of marker systems employed (Tanksley *et al.*, *J.P. Gustafson and R. Appels* (eds.). Plenum Press, New York, pp. 157-173 (1988)).
20 Consideration must be given to the source of parents (adapted vs. exotic) used in the mapping population. Chromosome pairing and recombination rates can be severely disturbed (suppressed) in wide crosses (adapted x exotic) and generally yield greatly reduced linkage distances. Wide crosses will usually provide segregating populations with
25 a relatively large number of polymorphisms when compared to progeny in a narrow cross (adapted x adapted).

An F₂ population is the first generation of selfing (self-pollinating) after the hybrid seed is produced. Usually a single F₁ plant is selfed to generate a population segregating for all the genes in Mendelian (1:2:1) pattern. Maximum genetic information is obtained
30 from a completely classified F₂ population using a codominant marker system (Mather, *Measurement of Linkage in Heredity*: Methuen and Co., (1938)). In the case of dominant markers, progeny tests (*e.g.*, F₃, BCF₂) are required to identify the heterozygotes, in order to classify the population. However, this procedure is often prohibitive because of the cost

and time involved in progeny testing. Progeny testing of F_2 individuals is often used in map construction where phenotypes do not consistently reflect genotype (e.g. disease resistance) or where trait expression is controlled by a QTL. Segregation data from progeny test populations e.g. F_3 or BCF_2) can be used in map construction. Marker-assisted selection can then be applied to cross progeny based on marker-trait map associations (F_2 , F_3), where linkage groups have not been completely disassociated by recombination events (i.e., maximum disequilibrium).

Recombinant inbred lines (RIL) (genetically related lines; usually $>F_5$, developed from continuously selfing F_2 lines towards homozygosity) can be used as a mapping population. Information obtained from dominant markers can be maximized by using RIL because all loci are homozygous or nearly so. Under conditions of tight linkage (i.e., about $<10\%$ recombination), dominant and co-dominant markers evaluated in RIL populations provide more information per individual than either marker type in backcross populations (Reiter. *Proc. Natl. Acad. Sci. (U.S.A.)* 89:1477-1481 (1992)). However, as the distance between markers becomes larger (i.e., loci become more independent), the information in RIL populations decreases dramatically when compared to codominant markers.

Backcross populations (e.g., generated from a cross between a successful variety (recurrent parent) and another variety (donor parent) carrying a trait not present in the former) can be utilized as a mapping population. A series of backcrosses to the recurrent parent can be made to recover most of its desirable traits. Thus a population is created consisting of individuals nearly like the recurrent parent but each individual carries varying amounts or mosaic of genomic regions from the donor parent. Backcross populations can be useful for mapping dominant markers if all loci in the recurrent parent are homozygous and the donor and recurrent parent have contrasting polymorphic marker alleles (Reiter *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 89:1477-1481 (1992)). Information obtained from backcross populations using either codominant or dominant markers is less than that obtained from F_2 populations because one, rather than two, recombinant gamete is sampled per plant. Backcross populations, however, are more informative (at low marker saturation) when compared to RILs as the distance between linked loci increases in RIL populations (i.e. about 0.15% recombination). Increased recombination can be beneficial for resolution of tight linkages, but may be undesirable in the construction of maps with low marker saturation.

Near-isogenic lines (NIL) (created by many backcrosses to produce a collection of individuals that is nearly identical in genetic composition except for the trait or genomic region under interrogation) can be used as a mapping population. In mapping with NILs, only a portion of the polymorphic loci is expected to map to a selected region.

5 Bulk segregant analysis (BSA) is a method developed for the rapid identification of linkage between markers and traits of interest (Michelmore *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 88:9828-9832 (1991)). In BSA, two bulked DNA samples are drawn from a segregating population originating from a single cross. These bulks contain individuals that are identical for a particular trait (resistant or susceptible to particular disease) or
10 genomic region but arbitrary at unlinked regions (*i.e.* heterozygous). Regions unlinked to the target region will not differ between the bulked samples of many individuals in BSA.

In an aspect of the present invention, one or more of the nucleic molecules of the present invention are used to determine the level (*i.e.*, the concentration of mRNA in a sample, *etc.*) in a plant (preferably canola, corn, *Brassica campestris*, *Brassica napus*,
15 oilseed rape, rapeseed, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, safflower, oil palm, flax or sunflower) or pattern (*i.e.*, the kinetics of expression, rate of decomposition, stability profile, *etc.*) of the expression of a protein encoded in part or whole by one or more of the nucleic acid molecule of the present invention (collectively, the "Expression Response" of a cell or tissue).

20 As used herein, the Expression Response manifested by a cell or tissue is said to be "altered" if it differs from the Expression Response of cells or tissues of plants not exhibiting the phenotype. To determine whether a Expression Response is altered, the Expression Response manifested by the cell or tissue of the plant exhibiting the phenotype is compared with that of a similar cell or tissue sample of a plant not exhibiting the
25 phenotype. As will be appreciated, it is not necessary to re-determine the Expression Response of the cell or tissue sample of plants not exhibiting the phenotype each time such a comparison is made; rather, the Expression Response of a particular plant may be compared with previously obtained values of normal plants. As used herein, the phenotype of the organism is any of one or more characteristics of an organism (*e.g.* disease
30 resistance, pest tolerance, environmental tolerance such as tolerance to abiotic stress, male sterility, quality improvement or yield *etc.*). A change in genotype or phenotype may be transient or permanent. Also as used herein, a tissue sample is any sample that comprises

more than one cell. In a preferred aspect, a tissue sample comprises cells that share a common characteristic (e.g. Derived from root, seed, flower, leaf, stem or pollen etc.).

In one aspect of the present invention, an evaluation can be conducted to determine whether a particular mRNA molecule is present. One or more of the nucleic acid
5 molecules of the present invention are utilized to detect the presence or quantity of the mRNA species. Such molecules are then incubated with cell or tissue extracts of a plant under conditions sufficient to permit nucleic acid hybridization. The detection of double-stranded probe-mRNA hybrid molecules is indicative of the presence of the mRNA; the amount of such hybrid formed is proportional to the amount of mRNA. Thus, such probes
10 may be used to ascertain the level and extent of the mRNA production in a plant's cells or tissues. Such nucleic acid hybridization may be conducted under quantitative conditions (thereby providing a numerical value of the amount of the mRNA present). Alternatively, the assay may be conducted as a qualitative assay that indicates either that the mRNA is present, or that its level exceeds a user set, predefined value.

15 A number of methods can be used to compare the expression response between two or more samples of cells or tissue. These methods include hybridization assays, such as northern, RNase protection assays, and *in situ* hybridization. Alternatively, the methods include PCR-type assays. In a preferred method, the expression response is compared by hybridizing nucleic acids from the two or more samples to an array of nucleic acids. The
20 array contains a plurality of suspected sequences known or suspected of being present in the cells or tissue of the samples.

An advantage of *in situ* hybridization over more conventional techniques for the detection of nucleic acids is that it allows an investigator to determine the precise spatial population (Angerer *et al.*, *Dev. Biol.* 101:477-484 (1984); Angerer *et al.*, *Dev. Biol.*
25 112:157-166 (1985); Dixon *et al.*, *EMBO J.* 10:1317-1324 (1991)). *In situ* hybridization may be used to measure the steady-state level of RNA accumulation (Hardin *et al.*, *J. Mol. Biol.* 202:417-431 (1989)). A number of protocols have been devised for *in situ* hybridization, each with tissue preparation, hybridization and washing conditions (Meyerowitz, *Plant Mol. Biol. Rep.* 5:242-250 (1987); Cox and Goldberg, In: *Plant*
30 *Molecular Biology: A Practical Approach*, Shaw (ed.), pp. 1-35, IRL Press, Oxford (1988); Raikhel *et al.*, *In situ RNA hybridization in plant tissues*, In: *Plant Molecular Biology Manual*, vol. B9:1-32, Kluwer Academic Publisher, Dordrecht, Belgium (1989)).

In situ hybridization also allows for the localization of proteins within a tissue or cell (Wilkinson, *In Situ Hybridization*, Oxford University Press, Oxford (1992); Langdale, *In Situ Hybridization In: The Corn Handbook*, Freeling and Walbot (eds.), pp. 165-179, Springer-Verlag, New York (1994)). It is understood that one or more of the molecules of the invention, preferably one or more of the nucleic acid molecules or fragments thereof of
 5 the invention, one or more of the antibodies of the invention may be utilized to detect the level or pattern of a protein or mRNA thereof by *in situ* hybridization.

Fluorescent *in situ* hybridization allows the localization of a particular DNA sequence along a chromosome, which is useful, among other uses, for gene mapping,
 10 following chromosomes in hybrid lines, or detecting chromosomes with translocations, transversions or deletions. *In situ* hybridization has been used to identify chromosomes in several plant species (Griffor *et al.*, *Plant Mol. Biol.* 17:101-109 (1991); Gustafson *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 87:1899-1902 (1990); Mukai and Gill, *Genome* 34:448-452 (1991); Schwarzacher and Heslop-Harrison, *Genome* 34:317-323 (1991); Wang *et al.*, *Jpn.*
 15 *J. Genet.* 66:313-316 (1991); Parra and Windle, *Nature Genetics* 5:17-21 (1993)). It is understood that the nucleic acid molecules of the invention may be used as probes or markers to localize sequences along a chromosome.

Another method to localize the expression of a molecule is tissue printing. Tissue printing provides a way to screen, at the same time on the same membrane many tissue
 20 sections from different plants or different developmental stages (Yomo and Taylor, *Planta* 112:35-43 (1973); Harris and Chrispeels, *Plant Physiol.* 56:292-299 (1975); Cassab and Varner, *J. Cell. Biol.* 105:2581-2588 (1987); Spruce *et al.*, *Phytochemistry* 26:2901-2903 (1987); Barres *et al.*, *Neuron* 5:527-544 (1990); Reid and Pont-Lezica, *Tissue Printing: Tools for the Study of Anatomy, Histochemistry and Gene Expression*, Academic Press,
 25 New York, New York (1992); Reid *et al.*, *Plant Physiol.* 93:160-165 (1990); Ye *et al.*, *Plant J.* 1:175-183 (1991)).

One skilled in the art can refer to general reference texts for detailed descriptions of known techniques discussed herein or equivalent techniques. These texts include *Current Protocols in Molecular Biology* Ausubel, *et al.*, eds., John Wiley & Sons, N.Y. (1989), and
 30 supplements through September (1998), *Molecular Cloning, A Laboratory Manual*, Sambrook *et al.*, 2nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, New York (1989), *Genome Analysis: A Laboratory Manual 1: Analyzing DNA*, Birren *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1997); *Genome Analysis: A Laboratory*

Manual 2: *Detecting Genes*, Birren *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1998); *Genome Analysis: A Laboratory Manual 3: Cloning Systems*, Birren *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1999); *Genome Analysis: A Laboratory Manual 4: Mapping Genomes*, Birren *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1999); *Plant Molecular Biology: A Laboratory Manual*, Clark, Springer-Verlag, Berlin, (1997), *Methods in Plant Molecular Biology*, Maliga *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1995). These texts can, of course, also be referred to in making or using an aspect of the invention. It is understood that any of the agents of the invention can be substantially purified and/or be biologically active and/or recombinant.

Having now generally described the invention, the same will be more readily understood through reference to the following examples that are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

Example 1.

Identification and characterization of mutant *hdt2 Arabidopsis thaliana*, ecotype Landsberg plants.

Mutagenized (M_2) seeds of *Arabidopsis thaliana*, ecotype Landsberg are obtained both by purchase from Lehle Seeds (Round Rock, Texas, U.S.A.) and by standard EMS mutagenesis methodology. The M_2 plants are grown from the M_2 seeds in greenhouse conditions with one plant per 2.5 inch pot. The resulting M_3 seeds are collected from individual M_2 plants and analyzed for tocopherol levels.

Seeds from approximately 10,000 M_3 lines of *Arabidopsis thaliana*, ecotype Landsberg or Col-O are analyzed for individual tocopherol levels using the following procedure. Five milligrams of seeds from individual plants are ground to a fine powder using a 1/8" steel ball bearing and vigorous shaking. 200 Microliters of 99.5% ethanol/0.5% pyrogallol is added, mixed for 30 seconds and allowed to incubate at 4°C for 1h. 50 Microgram/ml of tocol (Matreya, Inc., Pleasant Gap, PA) is added to each sample as an injection standard. To remove debris following centrifugation, the supernatant is filtered (PVDF 0.45 μ m, Whatman). The filtrate is then analyzed for tocopherol content using high performance liquid chromatography (HPLC) using an isocratic gradient of 90% hexane/10% methyl-t-butyl ether with a Zorbax silica column (4.6 x 250 mm, Agilent Technologies, Atlanta, GA) and fluorescence detection (model 2790 HPLC with model 474

detector; Waters Corporation, Bedford, MA) (excitation at 290 nm, emit at 336 nm, 30 nm bandpass and slits). Levels of α , β , γ , and δ -tocopherol are measured in addition to tocol, the injection standard. Individual plant lines that have δ -tocopherol levels higher than wild type are reanalyzed in the next generation (M4), to confirm their inheritability. Five

5 *Arabidopsis* high δ -tocopherol (hdt) mutants possessing increased levels of δ -tocopherols, as compared to wild type, are isolated.

Table 1 below shows the percentage, on a dry weight basis, of δ -tocopherol levels and the relative increases over the appropriate wild type parental ecotype for each of the six mutants. The results show that the six mutants have significant increases in δ -tocopherol

10 levels when compared to the corresponding wild type control. The magnitude of the increases ranged from 2-25 fold.

Table 1

Mutant	WT ecotype	Delta Composition	Increase over WT
hdt2	Ler	48%	25 fold
hdt6	Col-0	45%	20 fold
hdt9	Col-0	6%	2 fold
hdt10	Ler	25%	7 fold
hdt16	Col-0	50%	17 fold

Example 2.

Identification and sequencing of the mutant hdt2 gene in the *Arabidopsis thaliana*, Landsberg *erecta* (Ler) high δ -tocopherol mutants.

15 Using map-based cloning techniques (see, for example, USSN 09/803,736, Plant Polymorphic Markers and Uses Thereof, filed March 12, 2001) the mutant *hdt2* gene is mapped to chromosome 3 telomeric marker T12C14_1563 at 85 cM. This region contains approximately 60 predicted genes. Our analysis of the genes in this region revealed that one of the genes, MAA21_40, possesses homology to known ubiquinone

20 methyltransferases. Based on this homology and the prediction that MAA21_40 is targeted to the chloroplast, this gene is determined to be likely to contain the mutation responsible for the high δ -tocopherol phenotype in hdt2 mutants. The sequences of the MAA21_40 gene locus in the wild types and hdt2 mutants are PCR amplified, and determined by

standard sequencing methodology. The gene locus, in each case, is amplified using the sequencing primers as described below:

Primer Pair Name MAA21_40_1

Forward Primer TGTAACGACGGCCAGTTGCTGAAAGTTGAAAAGAGCAA (SEQ ID NO: 55)

5 Reverse Primer CAGGAAACAGCTATGACCCAATTTGATCAATGTTCCACGA (SEQ ID NO: 56)

Primer Pair Name MAA21_40_2

Forward Primer TGTAACGACGGCCAGTAGCTATGCGGATTGATGGTC (SEQ ID NO: 57)

Reverse Primer CAGGAAACAGCTATGACCTCCTCTGGGAACTCTAGCA (SEQ ID NO: 58)

Primer Pair Name MAA21_40_3

10 Forward Primer TGTAACGACGGCCAGTTGCTGACTTGCGAGTTTTTG (SEQ ID NO: 59)

Reverse Primer CAGGAAACAGCTATGACCCCTGTCAACAACCCCTTCTC (SEQ ID NO: 60)

Primer Pair Name MAA21_40_4

Forward Primer TGTAACGACGGCCAGTCCACAAGAGGGGTTTACAATG (SEQ ID NO: 61)

Reverse Primer CAGGAAACAGCTATGACCACCCAACCTTCTGGCTCTCT (SEQ ID NO: 62)

15 Primer Pair Name MAA21_40_5

Forward Primer TGTAACGACGGCCAGTGGTCTTTGGGAACGATCTGA (SEQ ID NO: 63)

Reverse Primer CAGGAAACAGCTATGACCAGGGAAGCGTACAGGGTTCT (SEQ ID NO: 64)

Primer Pair Name MAA21_40_6

Forward Primer TGTAACGACGGCCAGTCCTCTTGAGCTGAACGTCCT (SEQ ID NO: 65)

20 Reverse Primer CAGGAAACAGCTATGACCGGCGGAAGTGGTTTCACTAC (SEQ ID NO: 66)

Primer Pair Name MAA21_40_7

Forward Primer TGTAACGACGGCCAGTTGTCAGCATAATCGGTTGGA (SEQ ID NO: 67)

Reverse Primer CAGGAAACAGCTATGACCTCCCCAAAGGTTTAGGTTCC (SEQ ID NO: 68)

Primer Pair Name MAA21_40_8

25 Forward Primer TGTAACGACGGCCAGTAAGCCTCCTTCTGTGCTGA (SEQ ID NO: 69)

Reverse Primer CAGGAAACAGCTATGACCCGACTTTTCCCTTCCATTTG (SEQ ID NO: 70)

Primer Pair Name MAA21_40_9

Forward Primer TGTAACGACGGCCAGTTGGAGGTTCTGGGTAAGTGA (SEQ ID NO: 71)

Reverse Primer CAGGAAACAGCTATGACCCATCCTCTCGCTAGCAGGTC (SEQ ID NO: 72)

30 Primer Pair Name MAA21_40_10

Forward Primer TGTAACACGACGGCCAGTGGAAACCAGGGGAACCTAAAC (SEQ ID NO: 73)

Reverse Primer CAGGAAACAGCTATGACCGCCGTGAGAAACAGACTCCT (SEQ ID NO: 74)

Primer Pair Name MAA21_40_11

Forward Primer TGTAACACGACGGCCAGTCAAATGGAAGGGAAAAGTCG (SEQ ID NO: 75)

5 Reverse Primer CAGGAAACAGCTATGACCGATCCAAAGAGAACCCAGCA (SEQ ID NO: 76)

The following Polymerase Chain Reaction (PCR) mixture is prepared for each primer pair:

PCR mixture:

- 5 µl 10X Taq Buffer
- 10 5 µl 25mM MgCl₂
- 4 µl 10mM dNTPs
- 2 µl Template DNA
- 0.5 µl Taq Gold
- 5 µl F/R Sequencing Primers
- 15 28.5 µl dH₂O

The PCR amplification is carried out using the following Thermocycler program:

- 1. 94 °C for 10 minutes
- 2. 94 °C for 15 seconds
- 3. 56 °C for 15 seconds
- 20 4. 72 °C for 1 minute, 30 seconds
- 5. Repeat Steps 2 through 4 an additional 44 times
- 6. 72 °C for 10 minutes
- 7. Hold at 4 °C

- 25 The resulting PCR products are sequenced using standard sequencing methodologies.

- The wild type Col-0 genomic sequence for the MAA21_40 locus is set forth in SEQ ID NO: 1. The wild type Ler genomic sequence for the MAA21_40 locus is set forth in SEQ ID NO: 2. The wild type coding DNA and peptide sequence for Columbia and
- 30 Landsberg ecotypes are described in SEQ ID NOs: 15 and 16, respectively.

Once the sequences of the MAA21_40 gene from the hdt2 mutant are determined, they are compared to the sequence of the wild type gene. The high δ-tocopherol mutant identified as hdt2 is determined to have a MAA21_40 gene with the nucleic acid sequence

set forth in SEQ ID NO: 3. This sequence has a glutamate to lysine substitution at amino acid position 292, relative to the ATG of the *Arabidopsis* MAA21_40, as shown in the amino acid sequence of SEQ ID NO: 17.

Another high δ -tocopherol mutant, identified as hdt6, is determined to have a MAA21_40 gene with the nucleic acid sequence set forth in SEQ ID NO: 4. This sequence has a glutamate to a lysine substitution at amino acid 72, relative to the wild type *Arabidopsis* MAA21_40, as shown in the amino acid sequence of SEQ ID NO: 18.

Another high δ -tocopherol mutant, identified as hdt9 is determined to have a MAA21_40 gene with the nucleic acid sequence set forth in SEQ ID NO: 5. This sequence has a proline to a serine substitution at amino acid 13, relative to the *Arabidopsis* MAA21_40, as shown in the amino acid sequence of SEQ ID NO: 19.

Another high δ -tocopherol mutant, identified as hdt10 is determined to have a MAA21_40 gene with the nucleic acid sequence set forth in SEQ ID NO: 6 which encodes MAA21_40 with a aspartate to a asparagine substitution at amino acid 116, relative to the *Arabidopsis* MAA21_40, as shown in the amino acid sequence of SEQ ID NO: 20.

Another high δ -tocopherol mutant hdt16 is determined to have a MAA21_40 gene with the nucleic acid sequence set forth in SEQ ID NO: 7 which encodes MAA21_40 with a threonine to an isoleucine substitution at amino acid 94, relative to the *Arabidopsis* MAA21_40, as shown in the amino acid sequence of SEQ ID NO: 21.

Table 2 summarizes the mutations described above.

Table 2

Mutant	Nucleotide Mutation	Amino Acid Change
hdt2	G1041A	E292K
hdt6	G214A	E72K
hdt9	C37T	P13S
hdt10	G346A	D116N
hdt16	C281T	T94I

Example 3.

Identification of genes from various sources demonstrating homology to the tMT2 gene from *Arabidopsis thaliana*.

The protein sequence of tMT2 from *Arabidopsis thaliana* (NCBI General Identifier Number gi7573324) is used to search databases for plant sequences with homology to tMT2 using TBLASTN (Altschul *et al.*, *Nucleic Acids Res.* 25:3389-3402 (1997); see also www.ncbi.nlm.nih.gov/BLAST/). Nucleic acid sequences SEQ ID NO: 8 through 15 are found to have high homology with the *Arabidopsis* sequence..

>CPRI19219 *Brassica napus* tMT2 homolog 1 - LIB4153-013-R1-K1-B7
 ATGGCTTCTCTCATGCTCAACGGGGCCATCACCTTCCCCAAGGGATTAGGCTTCCCCGCTTCCAATCTACACG
 CCAGACCAAGTCTCCGCTGAGTCTCGTCTCAAACACAGCCACGCGGAGACTCTCCGTGGCGACAAGATGCAG
 10 CAGCAGCAGCAGCGTGTGCGCGTCAAGGCCATCTGCGCAGCCTAGGTTTCATCCAGCACAAGAAAGAGGCCTAC
 TGGTTCTACAGGTTCTGTCCATCGTGTACGACCACATCATCAATCCCGGCCACTGGACGGAGGATATGAGGG
 ACGACGCTCTCGAGCCTGCGGATCTGAGCCATCCGGACATGCGAGTTGTGACGCTCGGAGGCGGAACGGGTTT
 CACCACGCTGGGAATCGTCAAGACGGTGAAGGCTAAGAACGTGACGATTCTGGACCAGTCGCCGCATCAGCTG
 15 GCAAAGGCGAAGCAGAAGGAGCCGTTGAAGGAGTGCAAGATCGTTGAAGGAGATGCGGAGGATCTCCCTTTTC
 CTACTGATTATGCTGACAGATACGTCTCTGCTGGAAGCATTTGAGTACTGGCCCGACCCGAGAGGGGGATAAG
 GGAAGCGTACAGAGTTCTCAAGATCGGTGGGAAAGCATGTCTCATTGGCCCTGTCCACCCGACGTTTTGGCTT
 TCTCGTTTCTTTGCAGATGTGTGGATGCTTTTTCCCCAAGGAGGAGGAGTACATTGAGTGTTTCAAGAATGCTG
 GTTTCAAGGACGTTTCAAGAGGATTGGCCCCAAGTGGTACCGTGGTGTTCGAGGCACGGACTTATCAT
 20 GGGATGCTCTGTTACTGGTGTCAAACCTGCCTCTGGAGACTCTCCTCTCCAGCTTGGACCAAGGAAGAGGAC
 GTGGAGAAGCCTGTAAACAATCCTTTCTCCTTCTTGGGACGCTTCCTCTTGGGAACCTTAGCGGCTGCCTGGT
 TTGTGTTAATCCCAATCTACATGTGGATCAAGGATCAGATCGTTCCCAAGACCAACCCATCTGA (SEQ ID
 NO: 13)

>Protein sequence *Brassica napus* tMT2 homolog 1 - LIB4153-013-R1-K1-B7
 25 MASLMLNGAITFPKGLGFPASNLHARSPPLSLVSNATRRLSVATRCSSSSSVSASRPSAQPRFIQHKKEAY
 WFYRFLSIVYDHIINPGHWTEMDRDDALEPADLSHPDMRVVDVGGGTGFTTLGIVKTVKAKNVTILDQSPHQL
 AKAKQKEPLKECKIVEGDAEDLPFPTDYADRYVSAGSIEYWPDPQRGIREAYRVLKIGGKACLIGPVHPTFWL
 SRFFADVWMLFPKEEYIEWFKNAGFKDVQLKRIGPKWYRGVRRHGLIMGCSVTGVKPSGDSPLQLGPKEED
 VEKPVNNPFSFLGRFLLGTLAAAFVLIPIYMWIKDQIVPKDQPI (SEQ ID NO: 27)

>CPRI19220 *Brassica napus* tMT2 homolog 2 - LIB80-011-Q1-E1-E9
 ATGGCTTCTCTCATGCTCAACGGGGCCATCACCTTCCCCAAGGGATTAGGCTTCCCCGCTTCCAATCTACACG
 CCAGACCAAGTCTCCGCTGAGTCTCGTCTCAAACACAGCCACGCGGAGACTCTCCGTGGCGACAAGATGCAG
 35 CAGCAGCAGCAGCGTGTGCGCGTCAAGGCCATCTGCGCAGCCTAGGTTTCATCCAGCACAAGAAAGAGGCCTAC
 TGGTTCTACAGGTTCTGTCCATCGTGTACGACCACATCATCAATCCCGGCCACTGGACGGAGGATATGAGGG
 ACGACGCTCTCGAGCCTGCGGATCTGAGCCATCCGGACATGCGAGTTGTGACGCTCGGAGGCGGAACGGGTTT
 CACCACGCTGGGAATCGTCAAGACGGTGAAGGCTAAGAACGTGACGATTCTGGACCAGTCGCCGCATCAGCTG
 GCAAAGGCGAAGCAGAAGGAGCCGTTGAAGGAGTGCAAGATCGTGGAAGGAGATGCGGAGGATCTCCCTTTTC
 CTACTGATTATGCTGACAGATACGTCTCTGCTGGAAGCATTTGAGTACTGGCCCGACCCGAGAGGGGTATAAG
 40 GGAAGCGTACAGAGTTCTCAAGATCGGTGGGAAAGCATGTCTCATTGGCCCTGTCCACCCGACGTTTTGGCTT
 TCACGCTTCTTTGCAGATGTGTGGATGCTTTTCCCCAAGGAGGAGGAGTACATTGAGTGTTTCAAGAATGCTG
 GTTTCAAGGACGTTTCAAGAGGATTGGCCCCAAGTGGTACCGTGGTGTTCGAGGCACGGACTTATCAT
 GGGATGCTCTGTTACTGGTGTCAAACCTGCCTCTGGAGACTCTCCTCTCCAGCTTGGACCAAGGAAGAGGAC
 GTGGAGAAGCCTGTAAACAATCCTTTCTCCTTCTTGGGACGCTTCCTCTTGGGTACCCTAGCGGCTGCCTGGT
 45 TTGTGTTAATCCCAATCTACATGTGGATCAAGGATCAGATCGTTCCCAAGACCAACCCATCTGA (SEQ ID
 NO: 14)

> CPR 193223 *Oryza sativa* tMT2- LIB4371-041-R1-K1-F7
 ATGGCGATGGCCTCCTCCGCCTACGCCCCAGCGGGCGGCGTTGGCACCCACTCCGCGCCGGGAGGATCAGGC
 50 CGCCGCGCGGCCTCGGCTTCTCCACCACCACCACCAAGTCGAGGCCCTCGTGCTCACCAGGCGTGGGGGAGG
 CGCGCGCAACATCTCCGTGGCTCGGCTGAGGTGCGCGGCTCGTCTCGTCCGCGCGGCGAGGCCGATGTGCG
 CAGCCGCGGTTTATCCAGCACAAGAAGGAGGCGTTCTGGTTCTACCGCTTCTCTCCATCGTCTACGACCAG
 TCATCAACCCGGGCCACTGGACGGAGGACATGCGGGACGACGCCCTCGAGCCCGCCGACCTCTACAGCCGCAA
 GCTCAGGGTCTGTCGACGTCGCGCGGGACGGGGTTTACCACGCTCGGGATCGTCAAGCGCGTCGACCCGGAG
 55 AACGTCACGCTGCTCGACCAGTCCCCGACCAGCTCGAGAAGGCCCGGGAGAAGGAGGCCCTCAAGGGCGTCA
 CCATCATGGAGGGCGACGCCGAGGACCTCCCTTCCCCACCGACACCTTCGACCGCTACGTCTCCGCCGGCAG
 CATCGAGTATTGGCCCGATCCGCAGCGAGGAATCAAGGAAGCTTACAGGGTTTGGAGGCTTGGTGGAGTGGCT

TGCATGATTGGCCCCGTGCACCCAACCTTCTGGCTGTCTCGCTTTTTTCGCTGACATGTGGATGCTCTTCCCGA
AGGAAGAGGAGTATATTGAGTGGTTCAAAAAGGCAGGGTTCAAGGATGTCAAGCTCAAAAAGGATTGGACCAA
ATGGTACCGTGGTGTCCGAAGGCATGGCCTGATTATGGGATGCTCTGTGACGGGCGTCAAAAAGAGACATGGA
GACTCCCCCTTTCAGCTTGGTCCAAAGGTTGAGGATGTCAGCAAACCTGTGAATCCTATCACCTTCTCTTCC
5 GCTTCTCATGGGAACAATATGTGCTGCATACTATGTTCTGGTGCCTATCTACATGTGGATAAAGGACCAGAT
TGTGCCCAAAGGCATGCCGATCTAA (SEQ ID NO: 12)

> Protein translation *Oryza sativa* tMT2 - LIB4371-041-R1-K1-F7

10 MAMASSAYAPAGGVGTHSAPGRIRPPRGLGFSTTTTKSRPLVLRGGGGGNISVARLRCAASSSSAAARPMs
QPRFIQHKKEAFWFYRFLSIVYDHVINPGHWTEDMRDDALEPADLYSRKLRVVDVGGGTGFTTLGIVKRVDPE
NVTLLDQSPHQLEKAREKEALKGVTIMEGDAEDLPFPTDTFDYVSAGSIEYWPDPQRGIKEAYRVLRLGGVA
CMIGPVHPTFWLSRFFADMWMLFPKEEYIEWFKKAGFKDVKLRIGPKWYRGVRRHGLIMGCSVTGVKREHG
15 DSPLQLGPKVEDVSKPVNPITFLFRFLMGTICAAYYVLVPIYMWIKDQIVPKGMPI (SEQ ID NO: 26)

> CPR193225 and 193226 *Zea mays* tMT2- LIB3587-273-Q1-K6-C5/ LIB3600-046-
Q1-K6-G1

20 ATGGCGATGGCCTCCACCTACGCGCCGGGCGGAGGCGCGCGGGCGCTCGCGCAGGGTAGATGCAGGGTCCGCG
GTCCCGCGGGGCTGGGCTTCTCGGCCCTCCAAGCGCGCGGCTCCCCGCCCCCTCGCCCTCGCCCTCGC
CAGGCGGATGAGCAGCCCCGTGCGGGTGGGCGCCAGGCTGCGATGCGCGGCGTCTGCTCGTCCCCGCGGGCG
CGGCGCGCCACGGCGCGCGCTTCATCCAGCACAAGAAGGAGGCCTTCTGGTCTACCGCTTCTCTCCATCG
TGTACGACCACGTCATCAATCCGGGCCACTGGACCGAGGACATGCGCGACGACGCGCTGGAACCTGCCGACCT
CTTCAGCCGCCACCTCACGGTCGTCGACGTGCGCGGCGGCACGGGGTTCACCACGCTCGGCATCGTCAAGCAC
GTCAACCCGGAGAACGTCACGCTGCTCGACAGTCCCCGACAGCTCGACAAGGCCCGGCAGAAGGAGGCC
25 TCAAGGGGGTCAACATCATGGAGGGCGACGCCGAGGACCTCCCGTTCCCCACCGACTCCTTCGACCGATACAT
CTCCGCCGGCAGCATCGAGTACTGGCCAGACCCACAGCGGGGGATCAAGGAAGCCTACAGGGTCTGAGATTT
GGTGGGCTAGCTTGTGTGATCGGCCGGTCTACCCGACCTTCTGGCTGTCCCGCTTCTTCGCCGACATGTGGA
TGCTCTTCCCCAAGGAGGAAGAGTACATCGAGTGGTTCAAGAAGGCTGGGTTTAGGGATGTCAAGTGAAGAG
GATTGGACCGAAGTGGTACCGCGGTGTCCGAAGGCATGGCCTCATCATGGGCTGCTCCGTACAGGCGTCAAG
30 AGAGAGCGCGGTGACTCTCCCTTGGAGCTTGGTCCCAAGCGGAGGATGTCAGCAAGCCAGTGAATCCGATCA
CCTTCTCTTCCGCTTCCCTCGTAGGAACGATATGTGCTGCCTACTATGTTCTGGTGCCTATTTACATGTGGAT
AAAGGACCAGATCGTGCCAAAAGGCATGCCAATCTGA (SEQ ID NO: 8)

> Protein translation *Zea mays* tMT2- LIB3587-273-Q1-K6-C5/LIB3600-046-
Q1-K6-G1

35 MAMASTYAPGGGARALAQGRRCVRGPAGLGFLGPSKAAGLPRPLALALARRMSSPVAVGARLRCAASSSPAAA
RPATAPRFIQHKKEAFWFYRFLSIVYDHVINPGHWTEDMRDDALEPADLFSRHLTVVDVGGGTGFTTLGIVKH
VNPENVTLLDQSPHQLDKARQKEALKGVTIMEGDAEDLPFPTDSFDYISAGSIEYWPDPQRGIKEAYRVLRF
GGLACVIGPVYPTFWLSRFFADMWMLFPKEEYIEWFKKAGFRDVKLRIGPKWYRGVRRHGLIMGCSVTGVK
40 RERGDSPLELGPKAEDVSKPVNPITFLFRFLVGTICAAYYVLVPIYMWIKDQIVPKGMPI (SEQ ID NO:
22)

> CPR193234 *Glycine max* tMT2 - LIB3049-032-Q1-E1-G8

45 ATGGGTTTCAGTAATGCTCAGTGGAAGTGAAGGCTCACTCTCAGAACCTAACCGGGAACGGCTTAGGTTTCA
CTGGTTCGGATTTGCACGGTAAGAACTTCCCAAGAGTGAGTTTCGCTGCTACCACTAGTGCTAAAGTTCCCAA
CTTTAGAAGCATAGTAGTACCAAGTGATGTCTCGGCTTCCAGGCCAAGCTCGCAGCCAAGGTTCAATCAG
CACAAAAAAGAGGCCTTTTGGTTCTATAGGTTTCTCTCAATTGTGTATGACCATGTCAATTAACCTGGCCATT
GGACCGAGGACATGAGGGATGATGCCCTTGAACCGCTGATCTCAATGACAGGAACATGATTGTGGTGGATGT
50 TGGTGGCGGCACGGGTTTCAACCTCTTGGTATTGTCAAGCACGTGGATGCCAAGAATGTCACCATTTGAC
CAGTCACCCACACGCTCGCCAAGGCCAAGCAGAAGGAGCCACTCAAGGAATGCAAAATAATCGAAGGGGATG
CCGAGGATCTCCCTTTTCAAGTATTATGCCGATAGATATGTATCCGCAGGAAGTATTGAGTACTGGCCGGA
TCCACAGCGTGGCATCAAGGAGGCATACAGGGTTTTGAACTTGGAGGCAAAGCGTGTCTAATTGGTCCGGTC
TACCCAACATTTTGGTTGTACGTTTCTTTGCAGATGTTTGGATGCTTTTCCCAAGGAGGAAGATATATTG
AGTGGTTTTCAAGGACGGGTTAAGGACGTCCAATAAAAGGATTGGCCCAAATGGTATCGTGGGGTTTCG
55 CCGTCATGGCTTGATTATGGGTTGTTCAAGTACCGGTTTAAACCTGCATCTGGAGATTCTCCTTTGCAGCTT
GGTCCAAAGGAAGAAGATGTTGAAAGCCGTTAATCTTTTGTCTTTGCACTGCGCTTCGTTTTGGGTGCCT
TGGCAGCGACATGTTTGTGTTGTTTCTTATTTACATGTGGCTGAAAGATCAAGTTGTTCCCAAAGGTCAGCC
AATCTAA (SEQ ID NO: 11)

> Protein translation *Glycine max* tMT2 - LIB3049-032-Q1-E1-G8

MGSMVLSGTEKLTTLRLTLGNGLGFTGSDLHGKFNPRVSFAATTSKVPNFRSIVVPKCSVSASRPSSQPRFIQ
 HKKEAFWFYRFLSIVYDHVINPGHWTEDMRDDALEPADLNDNRNMIVVDVGGGTGFTTLGIVKHVDAKNVTILD
 QSPHQLAKAKQKEPLKECKIIIEGDAEDLPFRTDYADRYVSAGSIEYWPDQPGRIKEAYRVLKLGKACLIGPV
 YPTFWLSRFFADVWMLFPKEEEYIEWFQKAGFKDVQLKRIGPKWYRGVRRHGLIMGCSVTGVKPKASGDSPLQL
 5 GPKEEDVEKPVNPFVFLRFVLGALAATWFLVPIYMWLKDQVVPKGQPI (SEQ ID NO: 25)

>CPR193236 *Allium Porrum* - LIB4521-015-Q1-K1-D6
 ATGGCTTCCCTCCATGCTCAGCGGAGCAGAAAGCCTCTCAATGCTCCGAATCCACCACCAACCCAAACTCACCT
 TCTCGAGCCCATCCCTCCATTCCAAACCCACAAACCTCAAAATGGATCTCATCCCTTTTCGCCACCAAGCATCA
 10 AAAAACGAAAAAAGCTTCGATCTTTACATGCAGCGCTCCTCATCATCCCGACCTGCTTCTCAGCCGAGGTTT
 ATCCAGCACAAAGCAGGAGGCGTTCTGGTTCTACAGGTTCTGTCTGATAGTGTACGACCATGTGATAAACCCAG
 GGCACCTGGACCGAGGACATGAGAGACGATGCGTTGGAGCCAGCCGAGCTGTACGATTCCAGGATGAAGGTGGT
 GGACGTAGGAGGAGGAAGTGGGTTTACCACCTTGGGGATTATAAAGCACATCGACCCTAAAAACGTTACGATT
 CTGGATCAGTCTCCGCATCAGCTTGAGAAGGCTAGGCAGAAGGAGGCTTTGAAGGAGTGTACTATTGTTGAAG
 15 GTGATGCTGAGGATCTCCCTTTTCTACTGATACCTTCGATCGATATGTATCTGCTGGCAGCATAGAATACTG
 GCCAGACCCACAAAGAGGGATAAAGGAAGCATAACCGGGTTCTAAAACTGGGAGGCGTTGCTTGTGATAGGA
 CCCGTGCACCCTACCTTCTGGCTTTCCAGGTTCTTCGCCGACATGTGGATGTTGTTCCCCACCGAAGAAGAAT
 ACATAGAGTGGTTTAAAAAGGCCGGGTTCAAAGATGTGAAGTTGAAGAGGATTGGCCCCAAATGGTACCGTGG
 TGTGCGTAGACACGGGCTCATCATGGGCTGTTCCGTCAGTGGTTAAACGTCTCTCTGCTGACTCCCTCTT
 20 CAGCTTGGACCGAAGGCGGAGGATGTGAAGAAGCCGATCAATCCATTCTCGTTCCTTCTGCGCTTCATTTTGG
 GTACGATAGCAGCTACTTACTACGTTTGGTGCCGATATACATGTGGATAAAGGATCAGATTGTACCGAAAGG
 CCAGCCCATATGA (SEQ ID NO: 10)

>Protein translation *Allium Porrum* - LIB4521-015-Q1-K1-D6
 MASSMLSGAESLSMLRIHHQPKLTFSSPSLHSPKPTNLKMDLIPFATKHQKTKKASIFTCSASSSSRPASQPRF
 25 IQHKQEAFFWFYRFLSIVYDHVINPGHWTEDMRDDALEPAELYDSRMKVVDVGGGTGFTTLGIKIHIDPKNVTI
 LDQSPHQLAKAKQKEALKECTIVEGDAEDLPFPTDTFDYVSAGSIEYWPDQPGRIKEAYRVLKLGGAACCLIG
 PVHPTFWLSRFFADWMLFPTEEEYIEWFKKAGFKDVKLKRIGPKWYRGVRRHGLIMGCSVTGVKRLSGDSPL
 QLGPKAEDVKKPINPFSFLLRFILGTIAATYYVLVPIYMWIKDQIVPKGQPI (SEQ ID NO: 24)

>CPR204065 *Gossypium hirsutum* tMT2 - LIB3272-054-P1-K1-C11
 ATGGCTTCTTCCATGCTGAATGGAGCTGAAACCTTCACTCTCATCCGAGGTGTTACCCCAAAAAGTATTGGTT
 TTTTGGGGTCAGGTTTACATGGGAAACAGTTTCCAGTGCGGGTTAATCTACAGTCCGAAGATGTCCAGGGT
 AGGAACGACGATAGCCCCGAGGTGCAGCTTATCAGCGTCAAGGCCAGCTTCACAACCAAGATTTCATACAACAC
 30 AAAAAAGAGGCCTTTTGGTTCTACAGGTTCTCTCAATTGTCTATGACCATGTCTATAAACCCAGGTCACCTGGA
 CTGAAGACATGAGGGATGATGCACTTGAGCCGGCTGATCTCAATGACAGGGACATGGTAGTTGTAGATGTTGG
 TGGTGGAACTGGTTTCACTACTTTGGGTATTGTTGAGCATGTGGATGCTAAGAATGTTACAATCCTTGACCAA
 35 TCTCCTCACCAGGTTGCAAAGGCTAAACAGAGGAGCCTCTCAAGGAATGCAACATAATTGAAGGTGATGCAG
 AAGATCTTCTTTTCTACTGATTATGCCGATAGATATGTGTCTGCTGGAAGCATAGAGTACTGGCCAGACCC
 ACAACGGGGGATCAAGGAAGCATAACAGGTGTTGAAACAAGGAGGAAAAGCTTGCTTAATTGGTCTGTGTAC
 CCTACATTTTGGTTGTCTCGTTTCTTTGCAGACGTTTGGATGCTTTTCCCTAAGGAGGAAGAATATATAGAGT
 40 GGTTTGAAAAGGCTGGATTTAAGGATGTCCAACCTCAAAGGATTGGCCCTAAATGGTATCGTGGAGTTCGCCG
 ACATGGTTTGATCATGGGGTGCTCTGTAACCGGTGTTAAACCCGCATCTGGGGACTCTCCTTTGCAGCTTGGA
 CCTAAGGCAGAGGATGTATCAAAGCCGGTAAATCCGTTTGTATTTCTCTTACGCTTCATGTTGGGTGCCACTG
 CAGCAGCATATTATGTACTGGTTCCTATCTACATGTGGCTCAAAGATCAAATTGTACCAGAGGGTCAACCAAT
 45 CTAA (SEQ ID NO: 9)

>Protein translation *Gossypium hirsutum* tMT2 -LIB3272-054-P1-K1-C11
 MASSMLNGAETFTLIRGVTPKSIQFLGSLHGKQFSSAGLIYSPKMSRVGTTIAPRCSLSASRPASQPRFIQH
 KKEAFWFYRFLSIVYDHVINPGHWTEDMRDDALEPADLNDNRDMVVVDVGGGTGFTTLGIVQHVDAKNVTILDQ
 50 SPHQLAKAKQKEPLKECNIIIEGDAEDLPFPTDYADRYVSAGSIEYWPDQPGRIKEAYRVLKQGGKACLIGPVY
 PTFWLSRFFADVWMLFPKEEEYIEWFEKAGFKDVQLKRIGPKWYRGVRRHGLIMGCSVTGVKPKASGDSPLQLG
 PKAEDVSKPVNPFVFLRFMLGATAAAYYVLVPIYMWLKDQIVPEGQPI (SEQ ID NO: 23)

The protein sequence of tMT2 from *Arabidopsis thaliana* is compared against the
 tMT2 plant protein sequences listed above using BLASTP (Altschul *et al.*, *Nucleic Acids*
 55 *Res.* 25:3389-3402 (1997); see also www.ncbi.nlm.nih.gov/BLAST/). The calculated

protein identity of each sequence compared to the *Arabidopsis* sequence is shown in Figure 2. Also shown is a protein sequence alignment using the Pretty alignment program (Genetics Computer Group, Madison WI)(Figure 3).

Example 4

- 5 Preparation of constructs to direct the expression of the wild type tMT2 and mutant tMT2 gene sequences of *Arabidopsis thaliana* and tMT2 gene sequences from other crop plant species in a prokaryotic expression system.

A computer program is used to predict the chloroplast targeting peptide cleavage site of the plant tMT2 protein ("ChloroP", Center for Biological Sequence Analysis, 10 Lyngby, Denmark). The result of the search is as follows:

Name	Length	Score	cTP	CS-score	cTP-length
<i>Arabidopsis</i>	338	0.585	Y	6.467	51

Based on this information, the tMT2 protein from *Arabidopsis thaliana*, ecotype Landsberg is engineered to remove the predicted chloroplast target peptide to allow for the expression of the mature protein in *E. coli*. In order for these proteins to be expressed in a prokaryotic expression system, an amino terminal methionine is required. To make the addition of a 5' ATG the tMT2 coding sequence is amplified from cDNA of wild type and 15 the high δ -tocopherol hdt6, and hdt16 mutant lines of *Arabidopsis thaliana*, ecotype Columbia, and the high δ -tocopherol hdt2 and hdt10 mutant lines of *Arabidopsis thaliana*, ecotype Landsberg.

PolyA⁺ RNA is isolated from each source using an adapted biotin/streptavidin 20 procedure based on the "mRNA Capture Kit" by Roche Molecular Biochemicals (Indianapolis, IN). A young plantlet, approximately 1 cm tall, with root tissue removed is homogenized in CTAB buffer (50mM Tris-HCl pH 9, 0.8M NaCl, 0.5% CTAB, 10mM EDTA), extracted with chloroform, and pelleted with centrifugation. As specified by the manufacturer's instructions, polyA⁺ RNA in the soluble fraction is hybridized to biotin- 25 labeled oligo-dT, immobilized on streptavidin-coated PCR tubes and washed. The first strand cDNA is synthesized using the "1st strand cDNA synthesis kit for RT-PCR" (Roche Molecular Biochemicals) in a 50 μ l volume according to the manufacturer's protocol. Following the cDNA synthesis, the soluble contents of the tube are replaced with equal volume amplification reaction mixture. The components of the mixture at final 30 concentration consist of:

- 1X Buffer 2 (Expand™ High Fidelity PCR System, Roche Molecular Biochemicals)
- 200 μ M dNTPs
- 300nM each synthetic oligonucleotide primers;

5 #17180 FORWARD-NcoI
 5' GGGGACAAGTTTGTACAAAAAAGCAGGCTTAGAAGGAGATAGAACCATGGCTACTAGATGCA
 GCAGCAGCAGC 3' (SEQ ID NO: 79) and
 #17181 REVERSE-Sse8387I
 5' GGGGACCACTTTGTACAAGAAAGCTGGGTCCTGCAGGTCAGATGGGTTGGTCTTTGGGAACG
 10 3' . (SEQ ID NO: 78)

Each primer contains regions for GATEWAY™ cloning (Life Technologies Division, Invitrogen Corporation) as well as conventional restriction enzyme sites.

- 0.4 μ l Expand™ High Fidelity Polymerase (Roche Molecular Biochemicals)

15 Constructs are also prepared to direct expression of the engineered *Brassica napus*, *Oryza sativa*, *Zea mays*, *Glycine max*, *Allium Porrum*, and *Gossypium hirsutum* tMT2 sequences in a prokaryotic expression vector. The mature protein coding region of each tMT2 with the aminoterminal methionine, as described above, is amplified from plasmid DNA using the following oligonucleotide primers in the polymerase chain reaction.

20 The mature *Brassica napus* tMT2 coding sequence is amplified from LIB4153-013-R1-K1-B7 (SEQ ID NO: 13) using the synthetic oligonucleotide primers:

Brassica forward (17509)
 GGGACAAGTTTGTACAAAAAAGCAGGCTTAGAAGGAGATAGAACCATGGCGACAAGATGCAGCAGCAGCAGCA
 G (SEQ ID NO: 77) .

25 *Brassica* reverse (17181)
 GGGGACCACTTTGTACAAGAAAGCTGGGTCCTGCAGGTCAGATGGGTTGGTCTTTGGGAACG (SEQ ID
 NO: 78) .

The mature *Oryza sativa* tMT2 coding sequence is amplified from LIB4371-041-R1-K1-F7 (SEQ ID NO: 12) using the synthetic oligonucleotide primers:

30 Rice forward (17512)
 GGGACAAGTTTGTACAAAAAAGCAGGCTTAGAAGGAGATAGAACCATGCGGCTGAGGTGCGCGGCGTCGTCG
 (SEQ ID NO: 79) .

Rice reverse (17513)

GGGGACCACTTTGTACAAGAAAGCTGGGTCCTGCAGGTTAGATCGGCATGCCTTTGGGCAC (SEQ ID NO: 80).

The mature *Zea mays* tMT2 coding sequence is amplified from LIB3587-273-Q1-

5 K6-C5 (SEQ ID NO: 8) using the synthetic oligonucleotide primers:

Corn forward (17510)

GGGACAAGTTTGTACAAAAAGCAGGCTTAGAAGGAGATAGAACCATGAGGCTGCGATGCGGGCGTCGTCG (SEQ ID NO: 81).

Corn reverse (17511)

10 GGGGACCACTTTGTACAAGAAAGCTGGGTCCTGCAGGTCAGATTGGCATGCCTTTTGGCACG (SEQ ID NO: 82).

The mature *Glycine max* tMT2 coding sequence is amplified from LIB3049-032-Q1-E1-G8 (SEQ ID NO: 11) using the synthetic oligonucleotide primers:

Soy forward (17516)

15 GGGACAAGTTTGTACAAAAAGCAGGCTTAGAAGGAGATAGAACCATGGTACCCAAGTGTAGTGTCTCGGC (SEQ ID NO: 83).

Soy reverse (17517)

GGGGACCACTTTGTACAAGAAAGCTGGGTCCTGCAGGTTAGATTGGCTGACCTTTGGGAAC (SEQ ID NO: 84).

20 The mature *Allium Porrum* tMT2 coding sequence is amplified from LIB4521-015-Q1-K1-D6 (SEQ ID NO: 10) using the synthetic oligonucleotide primers:

Leek forward (17518)

GGGACAAGTTTGTACAAAAAGCAGGCTTAGAAGGAGATAGAACCATGATCTTTACATGCAGCGCGTCCT (SEQ ID NO: 85).

25 Leek reverse (17519)

GGGGACCACTTTGTACAAGAAAGCTGGGTCCTGCAGGTCATATGGGCTGGCCTTTTCGGTAC (SEQ ID NO: 86).

The mature *Gossypium hirsutum* tMT2 coding sequence is amplified from LIB3272-054-P1-K1-C11 (SEQ ID NO: 9) using the synthetic oligonucleotide primers:

Cotton forward (17514)

GGGACAAGTTTGTACAAAAAGCAGGCTTAGAAGGAGATAGAACCATGGCCCCGAGGTGCAGCTTATCAGCG

(SEQ ID NO: 87).

Cotton reverse (17515)

5 GGGGACCACTTTGTACAAGAAAGCTGGGTCCTGCAGGTTAGATTGGTTGACCCTCTGGTAC (SEQ ID NO: 88).

The components of each 100 μ l PCR reaction at final concentration consisted of:

- 0.5 μ l plasmid DNA diluted 1:20 with water
- 1X Buffer 2 (Expand™ High Fidelity PCR System, Roche Molecular
- 10 Biochemicals)
- 200 μ M dNTPs
- 300nM each, synthetic oligonucleotide primers
- 0.8 μ l Expand™ High Fidelity Polymerase (Roche Molecular Biochemicals)

The tMT2 gene from each source is PCR amplified for 30 cycles using the
15 following "touchdown" cycling profile. For each reaction the reaction mix is pre-incubated for 5 minutes at 95°C, during which the polymerase is spiked in. The product is then amplified for 15 cycles, each cycle consisting of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, and elongation at 72°C for 1.5 minutes. The annealing temperature is decreased by 1°C per cycle for each of the previous 15 cycles. An additional 15 cycles
20 follow, consisting of 94°C for 30 seconds, 45°C for 30 seconds, and 72°C for 1.5 minute, followed by a 7 minute hold at 72°C. The resulting amplification product is visualized as a clean band of the appropriate size for each species on a 0.8% agarose gel.

The resulting PCR products are subcloned into pDONR™201 (Life Technologies, A Division of Invitrogen Corp., Rockville, MD) using the GATEWAY cloning system
25 (Life Technologies, A Division of Invitrogen Corp., Rockville, MD).

To verify that no errors are introduced by the PCR amplification, the double stranded DNA sequence is obtained using standard sequencing methodology. The tMT2 sequences are then recombined behind the T7 promoter in the prokaryotic expression vector pET-DEST42 (Life Technologies, A Division of Invitrogen Corp., Rockville, MD)
30 using the GATEWAY cloning system (Life Technologies, A Division of Invitrogen Corp., Rockville, MD).

The following sequences represent the mature amino acid sequences of the wild type and mutant genes which may be expressed in *E. coli*, following the addition of an amino terminal methionine. The bolded and italicized amino acid residues represent the location of the substitution in each of the mutants.

- 5 Mature wildtype Arabidopsis tMT2 protein as expressed in *E. coli*:
 ATRCSSSSSVSSSRPSAQPRFIQHKKEAYWYRFLSIVYDHVINPGHWTE~~DMR~~DDALEPADLSHPDMRVVDVGG
 GTGFTTLGIVKTVKAKNVTILDQSPHQLAKAKQKEPLKECKIVEGDAEDLPFPTDYADRYVSAGSIEYWPDPQ
 RGIREAYRVLKIGGKACLIGPVYPTFWLSRFFSDVWMLFPKEEEYIEWFKNAGFKDVQLKRIGPKWYRGVRRH
 GLIMGCSVTGVKPSGDSPLQLGPKEEDVEKPVNNPFSFLGRFLLGTLAAAWFVLIPIYMWIKDQIVPKDQPI
 10 (SEQ ID NO: 28)
- Mature mutant hdt2 Arabidopsis tmt2 protein as expressed in *E. coli*
 ATRCSSSSSVSSSRPSAQPRFIQHKKEAYWYRFLSIVYDHVINPGHWTE~~DMR~~DDALEPADLSHPDMRVVDVGG
 GTGFTTLGIVKTVKAKNVTILDQSPHQLAKAKQKEPLKECKIVEGDAEDLPFPTDYADRYVSAGSIEYWPDPQ
 15 RGIREAYRVLKIGGKACLIGPVYPTFWLSRFFSDVWMLFPKEEEYIEWFKNAGFKDVQLKRIGPKWYRGVRRH
 GLIMGCSVTGVKPSGDSPLQLGPKEEDVEKPVNNPFSFLGRFLLGTLAAAWFVLIPIYMWIKDQIVPKDQPI
 (SEQ ID NO: 29)
- Mature mutant hdt6 Arabidopsis tmt2 protein as expressed in *E. coli*
 20 ATRCSSSSSVSSSRPSAQPRFIQHKKEAYWYRFLSIVYDHVINPGHWTE~~DMR~~DDALEPADLSHPDMRVVDVGG
 GTGFTTLGIVKTVKAKNVTILDQSPHQLAKAKQKEPLKECKIVEGDAEDLPFPTDYADRYVSAGSIEYWPDPQ
 RGIREAYRVLKIGGKACLIGPVYPTFWLSRFFSDVWMLFPKEEEYIEWFKNAGFKDVQLKRIGPKWYRGVRRH
 GLIMGCSVTGVKPSGDSPLQLGPKEEDVEKPVNNPFSFLGRFLLGTLAAAWFVLIPIYMWIKDQIVPKDQPI
 25 (SEQ ID NO: 30)
- Mature mutant hdt10 Arabidopsis tmt2 protein as expressed in *E. coli*
 ATRCSSSSSVSSSRPSAQPRFIQHKKEAYWYRFLSIVYDHVINPGHWTE~~DMR~~DDALEPADLSHPDMRVVNVGG
 GTGFTTLGIVKTVKAKNVTILDQSPHQLAKAKQKEPLKECKIVEGDAEDLPFPTDYADRYVSAGSIEYWPDPQ
 RGIREAYRVLKIGGKACLIGPVYPTFWLSRFFSDVWMLFPKEEEYIEWFKNAGFKDVQLKRIGPKWYRGVRRH
 30 GLIMGCSVTGVKPSGDSPLQLGPKEEDVEKPVNNPFSFLGRFLLGTLAAAWFVLIPIYMWIKDQIVPKDQPI
 (SEQ ID NO: 31)
- Mature mutant hdt16 Arabidopsis tmt2 protein as expressed in *E. coli*
 35 ATRCSSSSSVSSSRPSAQPRFIQHKKEAYWYRFLSIVYDHVINPGHWTE~~DMR~~DDALEPADLSHPDMRVVDVGG
 GTGFTTLGIVKTVKAKNVTILDQSPHQLAKAKQKEPLKECKIVEGDAEDLPFPTDYADRYVSAGSIEYWPDPQ
 RGIREAYRVLKIGGKACLIGPVYPTFWLSRFFSDVWMLFPKEEEYIEWFKNAGFKDVQLKRIGPKWYRGVRRH
 GLIMGCSVTGVKPSGDSPLQLGPKEEDVEKPVNNPFSFLGRFLLGTLAAAWFVLIPIYMWIKDQIVPKDQPI
 (SEQ ID NO: 32)
- 40 Mature *Brassica napus* tMT2 as expressed in *E. coli*
 ATRCSSSSSVSASRPSAQPRFIQHKKEAYWYRFLSIVYDHIINPGHWTE~~DMR~~DDALEPADLSHPDMRVVDVG
 GGTGFTTLGIVKTVKAKNVTILDQSPHQLAKAKQKEPLKECKIVEGDAEDLPFPTDYADRYVSAGSIEYWPDP
 QRGIREAYRVLKIGGKACLIGPVHPTFWLSRFFADVWMLFPKEEEYIEWFKNAGFKDVQLKRIGPKWYRGVRR
 HGLIMGCSVTGVKPSGDSPLQLGPKEEDVEKPVNNPFSFLGRFLLGTLAAAWFVLIPIYMWIKDQIVPKDQPI
 45 I. (SEQ ID NO: 33)
- Mature *Oryza sativa* tMT2 as expressed in *E. coli*
 RLRCAASSSSAAARPMSPQPRFIQHKKEAFWYRFLSIVYDHVINPGHWTE~~DMR~~DDALEPADLSRKLRVVDVG
 GGTGFTTLGIVKRVDPENVTLILDQSPHQLAKAKQKEALKGVTIMEGDAEDLPFPTDFTDRYVSAGSIEYWPDP
 50 QRGIREAYRVLRLGGVACMIGPVHPTFWLSRFFADMWMLFPKEEEYIEWFKNAGFKDVQLKRIGPKWYRGVRR
 HGLIMGCSVTGVKREHGDSPQLGPKEEDVSKPVNPITFLFRFLMGTICAAYYVLVPIYMWIKDQIVPKGMPI
 (SEQ ID NO: 34)
- 55 Mature *Zea mays* tMT2 as expressed in *E. coli*
 RLRCAASSSPAAARPATAPRFIQHKKEAFWYRFLSIVYDHVINPGHWTE~~DMR~~DDALEPADLSRHLTVVDVG
 GGTGFTTLGIVKHVNPENVTLILDQSPHQLDKARQKEALKGVTIMEGDAEDLPFPTDSFDRIYSAGSIEYWPDP
 QRGIREAYRVLRFGLACVIGPVYPTFWLSRFFADMWMLFPKEEEYIEWFKNAGFRDVQLKRIGPKWYRGVRR

HGLIMGCSVTGVKREGRGDSPLELGPKAEDVSKPVNPITFLFRFLVGTICAAYYVLVPIYMWIKDQIVPKGMP
(SEQ ID NO: 35)

Mature *Glycine max* tMT2 as expressed in *E. coli*

5 VPKCSVSASRPSSQPRFIQHKKEAFWFYRFLSIVYDHVINPGHWTEDMRDDALEPADLNDRNMIVVDVGGGTG
FTTLGIVKHVDAKNVTILDQSPHQLAKAKQKEPLKECKIIIEGDAEDLPFRTDYADRYVSAGSIEYWPDPQGI
KEAYRVLKLGKACLIGPVYPTFWLSRFFADVWMLFPKEEEYIEWFQKAGFKDVQLKRIGPKWYRGVRRHGLI
MGCSVTGVKPPASGDSPLQLGPKAEDVEKPVNPFVFLRFVLGALAATWFLVPIYMWLKDQVVPKGQPI
(SEQ ID NO: 36)

Mature *Allium Porrum* as expressed in *E. coli*

10 IFTCSASSSRPASQPRFIQHKQEAFFWFYRFLSIVYDHVINPGHWTEDMRDDALEPAELYDSRMKVVDVGGGT
GFTTLGIKHDIPKNVTILDQSPHQLAKAKQKEPLKECNIIEGDAEDLPFPTDTFDRYVSAGSIEYWPDPQGI
IKEAYRVLKLGKVACLIGPVHPTFWLSRFFADMWMLFPTEEYIEWFKAGFKDVKLKRIGPKWYRGVRRHGLI
15 IMGCSVTGVKRLSGDSPLQLGPKAEDVKKPINPFSLLRFILGTIAATYYVLVPIYMWIKDQIVPKGQPI
(SEQ ID NO: 37).

Mature *Gossypium hirsutum* tMT2 as expressed in *E. coli*

20 APRCSLSASRPASQPRFIQHKKEAFWFYRFLSIVYDHVINPGHWTEDMRDDALEPADLNDRDMVVVDVGGGTG
FTTLGIVQHVDAKNVTILDQSPHQLAKAKQKEPLKECNIIEGDAEDLPFPTDYADRYVSAGSIEYWPDPQGI
KEAYRVLKQGGKACLIGPVYPTFWLSRFFADVWMLFPKEEEYIEWFEKAGFKDVQLKRIGPKWYRGVRRHGLI
MGCSVTGVKPPASGDSPLQLGPKAEDVSKPVNPFVFLRFMLGATAAAYYVLVPIYMWLKDQIVPEGQPI
(SEQ ID NO: 38)

Example 5.

25 A 2-methylphytylplastoquinol methyltransferase enzymatic assay is performed on
the mature cloned genes expressed in *E. coli* to test for functionality of the encoded
proteins.

A culture is started by inoculating 100 mL of LB media with appropriate antibiotics
with an overnight starter culture of *E. coli* BL21(DE3) cells that is previously transformed
30 with prokaryotic expression constructs described in Example 4. The initial inoculation
results in an optical density of $OD_{600} = 0.1$ and the culture is grown at 25°C to a final
density of $OD_{600} = 0.6$. An amount corresponding to a final concentration of 0.4 mM IPTG
is added to induce protein expression, and the cells are then incubated at 25°C for 3 hours
until harvest.

35 The cells are chilled on ice for 5 minutes and then spun down at 5000 x g for 10
minutes. The cell pellet is stored at -80°C overnight after thoroughly aspirating off the
supernatant.

The cell pellet is thawed on ice and resuspended in 4mL of extraction buffer XB
(10mM HEPES-KOH pH7.8, 5mM DTT, 1mM AEBSF, 0.1 mM aprotinin, 1mg/ml
40 leupeptin). Cells are disrupted using a French press by making two passes through the
pressure cell at 20,000psi. Triton X-100 is added to a final concentration of 1% and the
extract is incubated on ice for one hour. The cell homogenate is then centrifuged at 5000 x
g for 10 minutes at 4°C.

The enzyme assays are run on the same day that the cells are extracted. The assays are run in 10mL polypropylene culture tubes with a final volume of 1mL. A reaction mixture consisting of the following is prepared and brought to a final volume of 950 μ L with distilled water.

5 **Reaction mixture:**

50mM Tris-HCl pH 8.0

5mM dithiothreitol (DTT, 100mM stock solution in water)

100 μ M 2-methylphytylplastoquinol (404g/mol)

0.5% Tween 80 (added directly to phytylplastoquinol after evaporating off solvent)

10 1.7 μ M 14 C-SAM (58 μ Ci/ μ mole)

2-Methyl-phytylplastoquinol and 2-methyl-geranylgeranylplastoquinol are synthesized as follows:

Fresh BF_3 -etherate (0.3ml) is added drop by drop to a solution of 400 mg methylquinol, 1000 mg isophytol in 10 ml dry dioxane. The mixture is stirred under N_2 in
15 the dark and is maintained at 50°C for 2 hours. The reaction mixture is hydrolyzed with ice, extracted with 3x15 ml petroleum ether/diethyl ether (1:1), the extract is washed several times with water to remove unused methylquinol, and dried with MgSO_4 . The solvent is evaporated off with a rotavapor to yield an oil like crude reaction product containing a mixture of methylplastoquinols. At this stage the reaction mixture is either separated into
20 various methylphytylplastoquinols by flash chromatography followed by HPLC purification or alternatively oxidized to yield the more stable methylplastoquinones. This is achieved by addition of a small amount of Ag_2O (200 mg) to the reaction product dissolved in diethyl ether for 1 hour. Removal of the Ag_2O by filtration provides the methylphytylplastoquinone mixture.

25 The synthesis of methylphytylplastoquinol as described above gives six isomers, namely 2'-cis and 2'-trans isomers of 2-methyl-3-phytylplastoquinol, 2-methyl-5-phytylplastoquinol 2-methyl-6-phytylplastoquinol. Purification of the six isomers is achieved by an initial separation of the methylphytylplastoquinol mixture into two bands on TLC (PSC-Fertigplatten Kieselgel 60 F₂₅₄₊₃₆₆, Merck, Darmstadt), using solvent system
30 petroleum ether:diethyl ether (7:3). The final purification of isomers of methylplastoquinols is achieved by semi-preparative HPLC.

HPLC is performed on a HP1100 series HPLC system consisting of HP G1329A Auto Sampler, HP G1311A Quaternary Pump, HP G1315A Diode Array Detector, HP G1321A Fluorescence Detector. Excitation is performed at 290 nm, emission is measured at 336 nm. In parallel, absorption is measured using a diode array detector set at 210 and 254 nm. The flow rate is kept at 5 mL/min. Plastoquinols are separated on isocratic HPLC using 90% Hexane:Methyl-Tertbutyl-Ether (90:10) on an Agilent Zorbax Silica 9.4 X 250 mm column.

Synthesis of 2-methyl-6-geranylgeranylplastoquinol is performed as the synthesis of 2-methyl-6-phytylplastoquinol, except geranyllinalool is used instead of isophytol for synthesis. The pure product is obtained from flash chromatography followed by repetitive TLC as described above.

To perform the methyltransferase assay 50 μ L of the cell extract is added to the assay mixture and mixed well. The reaction is initiated by adding 14 C-SAM (ICN) and incubating for one hour at 30°C in the dark. The reactions are then transferred to 15mL glass screw cap tubes equipped with Teflon coated caps. The reaction mixture is extracted with 4mL 2:1 CHCl₃/MeOH with 1mg/mL butylated hydroxy toluene (BHT) and mixed by vortex for 30 seconds. The tubes are centrifuged for 5 minutes to separate layers and the organic phase (bottom) is transferred to fresh 15mL glass tube. The CHCl₃ is evaporated off under a stream of nitrogen gas at 37°C for about 15 minutes. The residue is dissolved in 200 μ L of EtOH containing 1% pyrogallol and then mixed by vortex for 30 seconds. The resuspension is filtered into a brown LC vial equipped with an insert and analyzed by HPLC using a normal phase column (Agilent 4.6 x 250 mm Zorbax Sil, Agilent Technologies). The elution program is an isocratic flow of 10% methyl-*tert*-butyl-ether (MTBE) in hexane at 1.5 ml/minute for 12 minutes. Prior to each injection, a clean up run of 75% MTBE in hexane for 3 minutes is done, followed by a re-equilibration step of 10% MTBE in hexane for 3 minutes.

As a positive control, a pea chloroplast concentrate, which is known to have tMT2 activity, is prepared according to the procedure described by Arango and Heise, *Biochem J.* 336:531-533 (1998).

The results of these enzyme assays are shown in Figures 4-8. The series of HPLC chromatograms demonstrate that the cells transformed with the MT1 from *Anabaena*, which is known to have tMT2 activity (Figure 4) and the tMT2 from *Arabidopsis* (Figure

5) accumulate methylated products comigrating with a 2,3-dimethyl-5-phytylplastoquinone standard. The mutated tMT2 gene from *Arabidopsis* (hdt2) accumulated significantly less methylated products (Figure 6) than the wildtype tMT2 gene (Figure 5), showing that it has a decreased tMT2 activity. By way of comparison, the negative control where substrate is withheld from the cells transformed with the MT1 from *Anabaena* did not show a significant peak corresponding to the methylated products (Figure 7). Furthermore, the positive control of pea chloroplasts showed peaks corresponding to the methylated products obtained in the assays using *E. coli* extracts from strains harboring the MT1 and tMT2 expression constructs (Figure 8).

10 Expression and enzyme assay of crop tMT2 orthologs

tMT2 orthologs from *Brassica* (pMON67233), corn (pMON67234), leek (pMON67235), soybean (pMON67245), rice (pMON67232), and cotton (pMON67244), as well as the wild type *Arabidopsis* tMT2 (pMON67191), the hdt2 mutant (pMON67207), and the hdt10 mutant (pMON67243) are expressed as mature proteins in *E. coli* (Example 4). An *Anabaena* hdt2 ortholog is expressed from pMON67190. The *Anabaena* MT1 (pMON67174) and empty vector (pMON67179) are used as positive and negative controls, respectively. Cell growth, cell harvest, cell disruption, and enzyme assay are performed as described in Example 5. HPLC-purified 2-methyl-6-phytylplastoquinol is used as methyl group acceptor.

Table 3: 2-Methyl-6-phytylplastoquinol activity of recombinant expressed tMT2 genes

pMON #	Gene	Enzyme activity [μ U/mg protein]
67174	<i>Anabaena</i> MT1	6.5
67179	Plasmid control	< 1
67190	<i>Anabaena</i> tMT2 ortholog	< 1
67191	<i>Arabidopsis</i> tMT2	10
67207	<i>Arabidopsis</i> hdt2 mutant	1.1
67232	Rice tMT2 ortholog	4
67233	<i>Brassica</i> tMT2 ortholog	2
67234	Corn tMT2 ortholog	< 1
67235	Leek tMT2 ortholog	< 1
67243	<i>Arabidopsis</i> hdt10 mutant	< 1
67244	Cotton tMT2 ortholog	23.4
67245	Soy tMT2 ortholog	16.8

E. coli extracts expressing the *Anabaena* MT1, as well as mature proteins of the *Arabidopsis* tMT2, rice tMT2, cotton tMT2, and the soybean tMT2 are assayed as described in Example 5 using HPLC-purified 2-methyl-6-phytylplastoquinol, 2-methyl-5-phytylplastoquinol, or 2-methyl-3-phytylplastoquinol as methyl group acceptor. The assay demonstrates that tMT2 orthologs have a broader substrate range than the bacterial MT1 (Fig. 24).

Methyltransferase assays are performed using cell free *E. coli* extracts used in the experiments described above, expressing the *Anabaena* MT1, as well as the mature *Arabidopsis*, rice, cotton, and soybean tMT2s and 2-methyl-6-geranylplastoquinol, δ -tocopherol, γ -tocopherol, or β -tocopherol as methyl group accepting substrates. Enzyme activities are below the limit of detection with all four substrates.

Example 6:

Transformation and expression of a wild type *Arabidopsis* tMT2 gene in *Arabidopsis thaliana*.

The coding region of tMT2 is amplified from the EST clone Lib 3177-021-P1-K1-A3 (SEQ ID NO: 1) using the synthetic oligonucleotide primers;

#17286 FORWARD
GGGGACAAGTTTGTACAAAAAAGCAGGCTGCGGCCGCTGAACAATGGCCTCTTTGATGCTCAACG (SEQ ID NO: 89) and
17181 REVERSE
GGGGACCACTTTGTACAAGAAAGCTGGGTCTGCAGGTCAGATGGGTTGGTCTTTGGGAACG (SEQ ID NO: 90).

The amplification reaction consists of 1.0 μ l of EST template, 2.5 μ l 20X dNTPs, 2.5 μ l of each oligonucleotide primers, 5 μ l 10X PCR buffer, 35.75 μ l H₂O and 0.75 μ l Expand High Fidelity DNA Polymerase. PCR conditions for amplification are as follows:

- 1 cycle of 94° for 2 minutes, 10 cycles of 94°–15 seconds; 55°–30 seconds; and 72°– 1.5 minutes,
- 15 cycles of 94°–15 seconds; 55°–30 seconds; and 72°– 1.5 minutes adding 5 seconds to the 72° extension with each cycle,
- 1 cycle of 72° for 7 minutes.

After amplification, the samples are purified using a Qiagen PCR cleanup column (Qiagen Company, Valencia, California), suspended in 30 μ l water. The PCR reaction

products are separated on an agarose gel and visualized according to standard methodologies. The resulting PCR products are subcloned into pDONR™201 (Life Technologies, A Division of Invitrogen Corp., Rockville, MD) using the GATEWAY cloning system (Life Technologies, A Division of Invitrogen Corp., Rockville, MD). The resultant intermediate plasmid is named pMON67204 and the tMT2 sequence is confirmed by DNA sequencing using standard methodologies.

The wild type *Arabidopsis* tMT2 sequence is then cloned from the pMON67204 donor vector into the pMON67150 destination vector using the GATEWAY Technology kit (Life Technologies, a Division of Invitrogen Corporation, Rockville, MD.) according to the manufacturer's instructions. This destination vector is a GATEWAY compatible binary vector containing the napin cassette derived from pCGN3223 (described in U.S. Patent No. 5,639,790). The resultant expression vector is named pMON67205 (Figure 9) and is used to drive the expression of the tMT2 sequence in seeds.

The plant binary construct described above is used in *Arabidopsis thaliana* plant transformation to direct the expression of the tMT2 gene in the embryo. The binary vector construct is transformed into ABI strain *Agrobacterium* cells by the method of Holsters *et al.* *Mol. Gen. Genet.* 163:181-187 (1978). Transgenic *Arabidopsis thaliana* plants are obtained by *Agrobacterium*-mediated transformation of *Arabidopsis* wild type and the high δ -tocopherol mutants hdt2, hdt10, and hdt16 as described by Valverkens *et al.*, *Proc. Nat. Acad. Sci.* 85:5536-5540 (1988), Bent *et al.*, *Science* 265:1856-1860 (1994), and Bechtold *et al.*, *C.R. Acad. Sci., Life Sciences* 316:1194-1199 (1993). Transgenic plants are selected by sprinkling the transformed T₁ seeds directly onto soil and then vernalizing them at 4°C in the absence of light for 4 days. The seeds are transferred to 21°C, 16 hours light and sprayed with a 1:200 dilution of Finale (AgrEvo Environmental Health, Montvale, NJ) at 7 days and 14 days after seeding. Transformed plants are grown to maturity and the T₂ seed that is produced is analyzed for tocopherol content. The resulting tocopherol data shown in Tables 4 and 5 confirm a reduction of δ -tocopherol in favor of γ and α -tocopherol production in the high δ -tocopherol mutants and in wild type *Arabidopsis* lines. Tables 4 and 5 contain the results of HPLC analysis using the methodology (with minor

modifications) described in Savidge et al., *Plant Phys.* 129:321-332 (2000), Isolation and Characterization of Homogentisate Phytltransferase Genes from *Synechocystis* sp PCC 6803 and *Arabidopsis*.

5 Table 4 below details the results of the T₂ seed analysis.

Table 4

ng alpha toco./mg seed	ng beta toco./mg seed	ng gamma toco./mg seed	ng delta toco./mg seed	ng total toco./mg seed	Serial Number	Pedigree	Line #	% Delta	Average % Delta
5.88	0.00	529.64	18.87	554.39	69000076011	9979-AT00002-81:@.0001.	1	3.4	3.2
5.45	0.00	525.89	17.44	548.78	69000076009	9979-AT00002-81:@.0004.	4	3.2	
5.74	0.00	511.61	16.32	533.67	69000075994	9979-AT00002-81:@.0003.	3	3.1	
5.04	0.00	507.38	16.10	528.52	69000076023	9979-AT00002-81:@.0002.	2	3.0	
7.74	0.00	466.14	11.53	485.41	69000075463	67205-AT00002:0010.	10	T2 2.4	1.2
8.76	0.00	460.36	7.00	476.12	69000075540	67205-AT00002:0001.	1	T2 1.5	
8.33	0.00	445.02	6.71	460.06	69000075564	67205-AT00002:0004.	4	T2 1.5	
8.46	0.00	443.94	6.67	459.06	69000075502	67205-AT00002:0014.	14	T2 1.5	
11.13	0.00	447.27	6.35	464.75	69000075526	67205-AT00002:0016.	16	T2 1.4	
9.07	0.00	470.64	6.49	486.19	69000075552	67205-AT00002:0003.	3	T2 1.3	
8.10	0.00	422.89	5.82	436.81	69000075538	67205-AT00002:0002.	2	T2 1.3	
8.64	0.00	473.01	6.47	488.12	69000075603	67205-AT00002:0008.	8	T2 1.3	
9.25	0.00	488.63	6.43	504.32	69000075590	67205-AT00002:0007.	7	T2 1.3	
7.71	0.00	475.80	6.21	489.72	69000075588	67205-AT00002:0006.	6	T2 1.3	
7.77	0.00	458.67	5.71	472.15	69000075475	67205-AT00002:0011.	11	T2 1.2	
8.85	0.00	455.97	5.59	470.41	69000075576	67205-AT00002:0005.	5	T2 1.2	
10.27	0.00	349.67	3.05	362.98	69000075514	67205-AT00002:0015.	15	T2 0.8	
9.22	0.00	371.75	2.84	383.81	69000075499	67205-AT00002:0013.	13	T2 0.7	
8.68	0.00	348.97	2.53	360.18	69000075451	67205-AT00002:0009.	9	T2 0.7	
7.96	0.00	413.19	2.40	423.55	69000075487	67205-AT00002:0012.	12	T2 0.6	
7.00	0.00	277.36	286.49	570.84	69000077835	hdt2:@.0001.	1	50.2	49.7
6.57	0.00	273.89	278.92	559.38	69000077809	hdt2:@.0004.	4	49.9	
6.90	0.00	277.90	279.96	564.77	69000077811	hdt2:@.0003.	3	49.6	
6.93	0.00	275.20	273.89	556.01	69000077823	hdt2:@.0002.	2	49.3	
8.35	0.00	365.85	143.68	517.88	69000075639	67205-hdt2:0011.	11	T2 27.7	20.5
7.75	0.00	384.44	127.60	519.79	69000075689	67205-hdt2:0016.	16	T2 24.5	
7.05	0.00	358.91	105.17	471.13	69000075627	67205-hdt2:0010.	10	T2 22.3	
8.33	0.00	342.11	98.01	448.45	69000075665	67205-hdt2:0014.	14	T2 21.9	

ng alpha toco./mg seed	ng beta toco./mg seed	ng gamma toco./mg seed	ng delta toco./mg seed	ng total toco./mg seed	Serial Number	Pedigree	Line #	% Delta	Average % Delta	
6.73	0.00	410.18	112.97	529.88	69000075716	67205- hdt2:0006.	6 T2	21.3		
6.89	0.00	357.86	98.47	463.22	69000075704	67205- hdt2:0007.	7 T2	21.3		
6.85	0.00	352.48	96.71	456.04	69000075691	67205- hdt2:0008.	8 T2	21.2		
8.06	0.00	356.89	96.10	461.05	69000075754	67205- hdt2:0002.	2 T2	20.8		
7.60	0.00	311.53	82.55	401.68	69000075677	67205- hdt2:0015.	15 T2	20.6		
7.81	0.00	344.03	88.44	440.28	69000075615	67205- hdt2:0009.	9 T2	20.1		
7.50	0.00	368.30	88.66	464.46	69000075641	67205- hdt2:0012.	12 T2	19.1		
7.13	0.00	336.24	80.34	423.71	69000075728	67205- hdt2:0005.	5 T2	19.0		
7.78	0.00	345.26	81.26	434.30	69000075766	67205- hdt2:0001.	1 T2	18.7		
8.82	0.00	340.61	72.71	422.15	69000075730	67205- hdt2:0004.	4 T2	17.2		
8.11	0.00	418.69	81.01	507.81	69000075742	67205- hdt2:0003.	3 T2	16.0		
6.08	0.00	365.54	69.78	441.40	69000075653	67205- hdt2:0013.	13 T2	15.8		
3.36		262.76	180.18	446.30	69000157140	hdt16:@.0007.	Control	M5	40.4	38.2
3.36		290.12	177.76	471.24	69000157114	hdt16:@.0003.	Control	M5	37.7	
2.54		305.52	178.20	486.25	69000157099	hdt16:@.0005.	Control	M5	36.6	
4.93		248.24	67.78	320.95	69000156403	AT_G119:@.	PMON67205	R2	21.1	16.0
3.55		232.71	62.01	298.26	69000156667	AT_G36:@.	PMON67205	R2	20.8	
5.55		282.81	64.06	352.42	69000156679	AT_G37:@.	PMON67205	R2	18.2	
6.79		273.40	55.90	336.09	69000156617	AT_G31:@.	PMON67205	R2	16.6	
5.65		377.29	52.27	435.22	69000156631	AT_G33:@.	PMON67205	R2	12.0	
5.82		256.67	20.04	282.53	69000156655	AT_G35:@.	PMON67205	R2	7.1	
4.32		356.41	71.85	432.59	69000157037	hdt10:@.0001.	Control	M6	16.6	9.6
5.73		469.11	12.79	487.62	69000157049	hdt10:@.0002.	Control	M6	2.6	
3.39		308.41	27.44	339.24	69000156528	AT_G22:@.	PMON67205	R2	8.1	2.9
5.53		350.19	28.83	384.55	69000156592	AT_G29:@.	PMON67205	R2	7.5	
4.33		329.32	23.29	356.94	69000156489	AT_G18:@.	PMON67205	R2	6.5	
5.20		344.82	19.81	369.84	69000156566	AT_G26:@.	PMON67205	R2	5.4	
6.14		348.51	19.38	374.03	69000156453	AT_G15:@.	PMON67205	R2	5.2	
5.12		394.47	14.59	414.19	69000156578	AT_G27:@.	PMON67205	R2	3.5	
7.01		473.37	13.03	493.40	69000156530	AT_G23:@.	PMON67205	R2	2.6	
6.82		355.34	3.94	366.10	69000156580	AT_G28:@.	PMON67205	R2	1.1	
4.41		395.46	3.82	403.69	69000156477	AT_G17:@.	PMON67205	R2	0.9	
4.64		383.13	2.46	390.23	69000156542	AT_G24:@.	PMON67205	R2	0.6	
6.21		319.67	1.91	327.79	69000156465	AT_G16:@.	PMON67205	R2	0.6	
4.79		291.39	1.59	297.77	69000156441	AT_G14:@.	PMON67205	R2	0.5	
4.72		393.79	1.89	400.40	69000156491	AT_G19:@.	PMON67205	R2	0.5	
5.97		378.05	1.59	385.62	69000156516	AT_G21:@.	PMON67205	R2	0.4	
6.16		358.64	0.00	364.80	69000156554	AT_G25:@.	PMON67205	R2	0.0	

mp: indicates "metabolic profiling".

Table 5 below depicts the results of the analysis of T3 seed data from pMON67205 in hdt2 mutant lines.

Table 5

Crop	Biotype	Serial Number	mp:aT	mp:gT	mp:dT	total toco.	% delta	Gen	Pedigree	Construct
AT	SEED	69000357524	2	280	190	472	40.3	M7	hdt2:@.0001.0001.	
AT	SEED	69000357512	3	262	208	473	44.0	M7	hdt2:@.0001.0002.	
AT	SEED	69000357625	4	263	204	471	43.3	M7	hdt2:@.0001.0003.	
AT	SEED	69000357613	4	271	220	495	44.4	M7	hdt2:@.0001.0004.	
AT	SEED	69000357803	6	436	26	468	5.6	R3	67205-hdt2:0003.0001.	67205
AT	SEED	69000357790	4	336	149	489	30.5	R3	67205-hdt2:0003.0002.	67205
AT	SEED	69000357788	4	332	112	448	25.0	R3	67205-hdt2:0003.0003.	67205
AT	SEED	69000357776	3	334	140	477	29.4	R3	67205-hdt2:0003.0004.	67205
AT	SEED	69000357764	4	324	128	456	28.1	R3	67205-hdt2:0003.0005.	67205
AT	SEED	69000357598	3	363	97	463	21.0	R3	67205-hdt2:0003.0006.	67205
AT	SEED	69000357586	4	339	145	488	29.7	R3	67205-hdt2:0003.0007.	67205
AT	SEED	69000357574	4	372	99	475	20.8	R3	67205-hdt2:0003.0008.	67205
AT	SEED	69000357562	5	388	72	465	15.5	R3	67205-hdt2:0003.0009.	67205
AT	SEED	69000357550	4	341	63	408	15.4	R3	67205-hdt2:0013.0001.	67205
AT	SEED	69000357548	3	352	60	415	14.5	R3	67205-hdt2:0013.0002.	67205
AT	SEED	69000357536	4	386	54	444	12.2	R3	67205-hdt2:0013.0003.	67205
AT	SEED	69000358209	4	381	54	439	12.3	R3	67205-hdt2:0013.0004.	67205
AT	SEED	69000358196	6	413	73	492	14.8	R3	67205-hdt2:0013.0005.	67205
AT	SEED	69000358184	3	379	62	444	14.0	R3	67205-hdt2:0013.0006.	67205
AT	SEED	69000358172	5	382	63	450	14.0	R3	67205-hdt2:0013.0007.	67205
AT	SEED	69000358160	5	359	49	413	11.9	R3	67205-hdt2:0013.0008.	67205
AT	SEED	69000357601	4	371	4	379	1.1	R3	67205-hdt2:0013.0009.	67205

5 Example 7:

Method to prepare double gene constructs for expression in soybean and *Arabidopsis*.

Constructs are made containing promoters that provide seed-specific expression of the tMT2 gene alone and in combination with the GMT gene in soybean. Additionally the tMT2 gene is cloned behind the napin promoter and cloned into a binary vector with the HPT gene from *Arabidopsis* and in another double gene construct with the prenyltransferase (PT) gene (*slr1736*) from *Synechocystis* (pMON67224 and pMON67223 as shown in Figures 14 and 15, respectively).

Soybean Constructs

The wild type *Arabidopsis* tMT2 gene is cloned in between the 7S promoter and the pea SSU Rubisco 3' UTR in the vector pCGN3892 to create pMON67220 (Figure 10). This clone is then digested with Not I and the expression cassette is subcloned into the

plant binary expression vector pCGN11121 to create pMON67226 (Figure 11). This construct is used to transform soybean. Additionally, the *Arabidopsis* GMT between the 7S promoter and the pea SSU Rubisco 3' UTR is cut out from pMON36503 and then cloned into pMON67220 to create pMON67225 (Figure 12). These two genes under the control of 7S promoters are then cut out of pMON67225 with NotI and cloned into the Not site of pCGN11121 to create pMON67227 (Figure 13). This double gene construct is then used to transform soybean according to the procedure set forth in WO 00/61771 A3 on pages 99-100. Transformed plants are grown to maturity and seed that is produced is analyzed for total tocopherol content and composition.

The tocopherol data presented in Tables 3 and 5 demonstrate the reduction of β -tocopherol and more so, δ -tocopherol in favor of γ and α -tocopherol production in soybean seeds harboring a tMT2 expression construct. Tables 4 and 6 demonstrate a nearly complete (98% in the R0 generation) conversion of tocopherols into α -tocopherol in soybean seed harboring a double gene expression construct for tMT2 and a γ -methyltransferase.

Table 6 below depicts the results of the analysis of various soybean lines transformed with pMon67226 Soy. Tables 6 and 9 contain the results of HPLC analysis using the methodology (with minor modifications) described in Savidge et al., *Plant Phys.* 129:321-332 (2000), Isolation and Characterization of Homogentisate Phytltransferase Genes from *Synechocystis* sp PCC 6803 and *Arabidopsis*.

Table 6

Pedigree	% delta	% gamma	% alpha	% beta*	mp:aT	mp:bT	mp:gT	mp:dT	total toco.
A3244	22.90	63.97	10.44	2.69	31	8	190	68	297
A3244	22.85	64.24	10.26	2.65	31	8	194	69	302
A3244	22.88	64.38	10.46	2.29	32	7	197	70	306
A3244	23.08	64.21	10.37	2.34	31	7	192	69	299
A3244	22.97	64.19	10.47	2.36	31	7	190	68	296
GM_A28213:@	36.92	51.08	8.31	3.69	27	12	166	120	325
GM_A27926:@	27.51	62.72	7.46	2.31	29	9	244	107	389
GM_A27928:@	26.56	62.81	8.13	2.50	26	8	201	85	320
GM_A27993:@	25.70	62.29	9.50	2.51	34	9	223	92	358
GM_A27628:@	25.07	61.19	10.75	2.99	36	10	205	84	335
GM_A28069:@	24.66	58.56	13.01	3.77	38	11	171	72	292
GM_A27927:@	24.41	63.05	10.17	2.37	30	7	186	72	295
GM_A28930:@	24.14	63.01	10.03	2.82	32	9	201	77	319
GM_A28597:@	23.89	61.09	11.60	3.41	34	10	179	70	293
GM_A28077:@	23.73	65.76	8.47	2.03	25	6	194	70	295
GM_A28410:@	23.70	66.47	7.80	2.02	27	7	230	82	346

Pedigree	% delta	% gamma	% alpha	% beta*	mp:aT	mp:bT	mp:gT	mp:dT	total toco.
GM_A28212:@	23.37	63.91	10.06	2.66	34	9	216	79	338
GM_A28079:@	23.10	62.38	11.22	3.30	34	10	189	70	303
GM_A27992:@	23.05	52.42	19.70	4.83	53	13	141	62	269
GM_A28074:@	22.52	61.86	12.61	3.00	42	10	206	75	333
GM_A28931:@	20.66	63.28	13.44	2.62	41	8	193	63	305
GM_A28767:@	20.20	65.66	11.78	2.36	35	7	195	60	297
GM_A28598:@	20.14	61.09	15.02	3.75	44	11	179	59	293
GM_A28214:@	20.07	61.90	14.29	3.74	42	11	182	59	294
GM_A28062:@	19.80	64.09	13.09	3.02	39	9	191	59	298
GM_A28505:@	19.69	66.77	11.69	1.85	38	6	217	64	325
GM_A28067:@	18.18	62.55	15.64	3.64	43	10	172	50	275
GM_A28503:@	18.06	65.63	14.24	2.08	41	6	189	52	288
GM_A28408:@	17.97	64.75	14.58	2.71	43	8	191	53	295
GM_A28061:@	17.87	62.20	16.15	3.78	47	11	181	52	291
GM_A28504:@	17.73	62.06	16.67	3.55	47	10	175	50	282
GM_A28409:@	16.79	63.14	16.42	3.65	45	10	173	46	274
GM_A28060:@	16.16	68.35	13.80	1.68	41	5	203	48	297
GM_A28076:@	16.04	60.41	19.11	4.44	56	13	177	47	293
GM_A28066:@	15.36	59.73	20.48	4.44	60	13	175	45	293
GM_A29037:@	14.49	71.59	12.22	1.70	43	6	252	51	352
GM_A27855:@	13.64	74.68	10.39	1.30	32	4	230	42	308
GM_A27856:@	13.46	72.76	12.18	1.60	38	5	227	42	312
GM_A28081:@	11.11	76.85	10.80	1.23	35	4	249	36	324
GM_A27627:@	8.33	75.93	14.20	1.54	46	5	246	27	324
GM_A27932:@	8.13	81.33	9.94	0.60	33	2	270	27	332
GM_A27857:@	7.28	78.48	13.29	0.95	42	3	248	23	316
GM_A28073:@	7.22	67.70	23.37	1.72	68	5	197	21	291
GM_A27708:@	7.06	75.77	16.26	0.92	53	3	247	23	326
GM_A28059:@	6.99	77.57	14.71	0.74	40	2	211	19	272
GM_A27925:@	6.95	76.82	15.23	0.99	46	3	232	21	302
GM_A27859:@	6.83	77.34	14.39	1.44	40	4	215	19	278
GM_A28065:@	6.44	73.22	18.64	1.69	55	5	216	19	295
GM_A27931:@	6.33	78.92	13.86	0.90	46	3	262	21	332
GM_A28246:@	6.31	72.24	19.87	1.58	63	5	229	20	317
GM_A27994:@	6.29	79.02	13.99	0.70	40	2	226	18	286
GM_A27995:@	6.08	78.12	14.89	0.91	49	3	257	20	329
GM_A28075:@	5.61	73.60	19.14	1.65	58	5	223	17	303
GM_A28070:@	5.47	79.42	14.47	0.64	45	2	247	17	311
GM_A28068:@	4.76	75.85	18.71	0.68	55	2	223	14	294
GM_A28078:@	3.72	81.08	14.53	0.68	43	2	240	11	296
GM_A28080:@	3.69	73.06	21.77	1.48	59	4	198	10	271
GM_A28071:@	3.64	75.83	19.87	0.66	60	2	229	11	302
GM_A28058:@	3.51	82.16	13.74	0.58	47	2	281	12	342
GM_A28064:@	2.23	85.03	12.74	0.00	40	0	267	7	314
GM_A28599:@	1.47	82.65	15.88	0.00	54	0	281	5	340
GM_A27929:@	1.23	83.74	13.80	1.23	45	4	273	4	326
GM_A28063:@	1.22	74.62	23.55	0.61	77	2	244	4	327
GM_A28072:@	0.95	76.66	22.08	0.32	70	1	243	3	317
GM_A27930:@	0.68	79.05	20.27	0.00	60	0	234	2	296

Table 7 below sets forth the results of the analysis of various soybean lines transformed with pMON 67227.

Table 7

Pedigree	% alpha	% beta	% gamma	% delta	mp:aT	mp:bT	mp:gT	mp:dT	total toco.
A3244	10.4	2.7	64.0	22.9	31	8	190	68	297
A3244	10.3	2.6	64.2	22.8	31	8	194	69	302
A3244	10.5	2.3	64.4	22.9	32	7	197	70	306
A3244	10.4	2.3	64.2	23.1	31	7	192	69	299
A3244	10.5	2.4	64.2	23.0	31	7	190	68	296
GM_A27999:@.	9.5	2.5	62.9	25.2	31	8	205	82	326
GM_A28091:@.	10.5	3.1	61.9	24.5	31	9	182	72	294
GM_A28090:@.	11.3	2.7	63.0	22.9	33	8	184	67	292
GM_A28933:@.	14.4	2.1	65.8	17.7	48	7	219	59	333
GM_A28601:@.	15.7	3.1	62.4	18.8	45	9	179	54	287
GM_A27712:@.	60.4	2.5	26.9	10.2	171	7	76	29	283
GM_A27936:@.	60.6	20.4	13.8	5.2	163	55	37	14	269
GM_A28093:@.	67.2	3.3	21.2	8.3	203	10	64	25	302
GM_A27934:@.	75.4	3.1	16.5	5.0	196	8	43	13	260
GM_A28096:@.	79.1	3.4	12.5	5.0	253	11	40	16	320
GM_A27935:@.	88.5	2.7	6.9	1.9	231	7	18	5	261
GM_A27998:@.	89.6	2.5	6.0	1.9	285	8	19	6	318
GM_A27711:@.	91.4	3.3	4.3	1.0	276	10	13	3	302

5 Table 8 below sets for the results of the analysis of single seeds of soybean transformed with pMON 67226.

Table 8

Pedigree	% alpha	% beta*	% gamma	% delta	mp:aT	mp:bT	mp:gT	mp:dT	total toco.
GM_A27930:@.	12.2	3.4	64.1	20.3	29	8	152	48	237
GM_A27930:@.	21.7	0.0	77.9	0.4	55	0	197	1	253
GM_A27930:@.	15.0	0.0	84.0	1.0	46	0	257	3	306
GM_A27930:@.	22.4	0.0	76.8	0.8	58	0	199	2	259
GM_A27930:@.	13.9	0.0	85.7	0.4	33	0	204	1	238
GM_A27930:@.	21.7	0.0	77.6	0.7	63	0	225	2	290
GM_A27930:@.	21.7	0.0	77.6	0.8	55	0	197	2	254
GM_A27930:@.	25.7	0.0	74.0	0.4	68	0	196	1	265
GM_A28072:@.	22.4	0.0	76.8	0.8	57	0	195	2	254
GM_A28072:@.	31.3		67.6	1.2	80	0	173	3	256
GM_A28072:@.	22.8	0.0	76.5	0.7	64	0	215	2	281
GM_A28072:@.	17.6	0.0	81.5	1.0	55	0	255	3	313
GM_A28072:@.	20.0	0.0	78.9	1.1	55	0	217	3	275
GM_A28072:@.	35.0	0.0	64.6	0.4	97	0	179	1	277
GM_A28072:@.	31.5	0.0	68.1	0.4	80	0	173	1	254
GM_A28072:@.	16.4	0.0	82.6	1.0	51	0	257	3	311

Table 9 below sets forth the results of the analysis of single seeds of soybean transformed with pMON 67227.

Table 9

Pedigree	% alpha	% beta*	%gamma	% delta	mp:aT	mp:bT	mp:gT	mp:dT	total toco.
GM_A27711:@.	97.8	2.2	0.0	0.0	263	6	0	0	269
GM_A27711:@.	96.7	3.3	0.0	0.0	320	11	0	0	331
GM_A27711:@.	96.5	3.5	0.0	0.0	301	11	0	0	312
GM_A27711:@.	96.7	3.3	0.0	0.0	295	10	0	0	305
GM_A27711:@.	96.9	3.1	0.0	0.0	308	10	0	0	318
GM_A27711:@.	97.3	2.7	0.0	0.0	287	8	0	0	295
GM_A27711:@.	98.2	1.8	0.0	0.0	272	5	0	0	277
GM_A27711:@.	95.7	4.3	0.0	0.0	287	13	0	0	300
GM_A27935:@.	10.3	2.6	65.4	21.7	28	7	178	59	272
GM_A27935:@.	98.5	1.5	0.0	0.0	261	4	0	0	265
GM_A27935:@.	98.3	1.7	0.0	0.0	230	4	0	0	234
GM_A27935:@.	98.6	1.4	0.0	0.0	272	4	0	0	276
GM_A27935:@.	98.2	1.8	0.0	0.0	267	5	0	0	272
GM_A27935:@.	96.9	3.1	0.0	0.0	277	9	0	0	286
GM_A27935:@.	98.3	1.7	0.0	0.0	337	6	0	0	343
GM_A27935:@.	96.5	3.5	0.0	0.0	276	10	0	0	286
GM_A27998:@.	97.0	3.0	0.0	0.0	318	10	0	0	328
GM_A27998:@.	97.1	2.9	0.0	0.0	300	9	0	0	309
GM_A27998:@.	95.9	4.1	0.0	0.0	324	14	0	0	338
GM_A27998:@.	97.0	3.0	0.0	0.0	292	9	0	0	301
GM_A27998:@.	96.9	3.1	0.0	0.0	314	10	0	0	324
GM_A27998:@.	96.5	3.5	0.0	0.0	359	13	0	0	372
GM_A27998:@.	96.5	3.5	0.0	0.0	335	12	0	0	347
GM_A27998:@.	96.6	3.4	0.0	0.0	310	11	0	0	321
GM_A28096:@.	11.1	3.7	61.0	24.1	36	12	197	78	323
GM_A28096:@.	9.5	3.3	61.4	25.8	29	10	188	79	306
GM_A28096:@.	96.8	3.2	0.0	0.0	299	10	0	0	309
GM_A28096:@.	96.0	4.0	0.0	0.0	288	12	0	0	300
GM_A28096:@.	95.8	4.2	0.0	0.0	319	14	0	0	333
GM_A28096:@.	95.8	4.2	0.0	0.0	295	13	0	0	308
GM_A28096:@.	97.8	2.2	0.0	0.0	316	7	0	0	323
GM_A28096:@.	95.8	4.2	0.0	0.0	300	13	0	0	313

The * next to % beta in Tables 6 through 9 is a label to indicate that β -tocopherol comigrates with an unknown compound, making it difficult to quantify.

Arabidopsis double constructs

The tMT2 gene is cut out of the vector pMON67204 using the restriction enzymes Not I (blunt)/Pst I and then cloned into the napin shuttle vector pCGN3223 which is digested with Sal (blunt)/Pst I. This napin cassette containing the tMT2 gene is then cut out from this vector with *Not* I and the ends are filled in with dNTPs using a Klenow procedure. The resulting fragment is inserted into the vectors pMON16602 (digested with

PmeI) and pCGN10822 (digested with *SnaBI*) to make pMON67224 and pMON67223, respectively (Figures 14 and 15). The vectors pMON16602 and pCGN10822 are described in PCT application WO 0063391.

5 These double constructs express the tMT2 gene and the prenyltransferase from either *Arabidopsis* (HPT) or *Synechocystis* (*slr1736*) under the control of the napin seed-specific promoter. These constructs are used to transform *Arabidopsis* and transformed plants are grown to maturity, as detailed in Example 6. The resulting T₂ seed is analyzed for total tocopherol content and composition using analytical procedures described in Example 1.

What is claimed is:

1. A substantially purified nucleic acid molecule encoding a plant polypeptide having 2-methylphytylplastoquinol methyltransferase activity.
2. The substantially purified nucleic acid molecule of claim 1, wherein said
5 plant is selected from the group consisting of *Arabidopsis thaliana*, Columbia ecotype, *Arabidopsis thaliana*, Landsberg ecotype, corn, soybean, rice, *Allium*, *Brassica*, and *Gossypium*.
3. The substantially purified nucleic acid molecule of claim 1, wherein said nucleic acid molecule encodes a polypeptide molecule comprising an amino acid sequence
10 selected from the group consisting of SEQ ID NOs: 16 through 38.
4. The substantially purified nucleic acid molecule of claim 1, wherein said nucleic acid molecule encodes a mutant plant polypeptide having 2-methylphytylplastoquinol methyltransferase activity.
5. The substantially purified nucleic acid molecule of claim 4, wherein said
15 nucleic acid molecule is a mutant gene selected from the group consisting of hdt2, hdt6, hdt9, hdt10, and hdt16.
6. A substantially purified nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 3 through 14, and complements thereof.
- 20 7. A substantially purified plant polypeptide molecule having 2-methylphytylplastoquinol methyltransferase activity.
8. The substantially purified plant polypeptide molecule of claim 7, wherein said polypeptide molecule is native to an organism selected from the group consisting of *Arabidopsis thaliana*, Columbia ecotype, *Arabidopsis thaliana*, Landsberg ecotype, corn,
25 soybean, rice, *Allium*, *Brassica*, and *Gossypium*.
9. A transformed plant comprising an introduced nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof.

10. The transformed plant of claim 9, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, citrus, canola, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, chick peas, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

11. The transformed plant of claim 9, wherein said transformed plant produces a seed with one or both of an increased γ -tocopherol level and increased γ -tocotrienol level relative to a plant with a similar genetic background but lacking said introduced nucleic acid molecule.

12. The transformed plant of claim 9, wherein said nucleic acid molecule further comprises, in the 5' to 3' direction, a heterologous promoter operably linked to said nucleic acid sequence.

13. The transformed plant of claim 12, wherein said promoter is a seed specific promoter.

14. A transformed plant comprising an introduced nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, and 33 through 38.

15. The transformed plant of claim 14, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, citrus, canola, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

16. The transformed plant of claim 14, wherein said transformed plant produces a seed with one or both of an increased γ -tocopherol level and γ -tocotrienol level relative to

a plant with a similar genetic background but lacking said introduced nucleic acid molecule.

17. The transformed plant of claim 14, wherein said nucleic acid molecule further comprises, in the 5' to 3' direction, a heterologous promoter operably linked to said nucleic acid sequence.

18. The transformed plant of claim 17, wherein said promoter is a seed specific promoter.

19. A transformed plant comprising an introduced nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, and 33 through 38.

20. The transformed plant of claim 19, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, citrus, canola, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

21. A transformed plant comprising an introduced first nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof, and an introduced second nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA*, *slr1736*, *HPT*, *GMT*, *tocopherol cyclase*, *dxs*, *dxr*, *GGPPS*, *HPPD*, *AANT1*, *slr 1737*, *IDI*, *GGH*, complements thereof, a plant ortholog thereof and an antisense construct for homogentisic acid dioxygenase.

22. The transformed plant of claim 21, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, citrus, canola, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils,

grape, banana, tea, turf grasses, sunflower, soybean, chick peas, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

23. The transformed plant of claim 21, wherein said introduced second nucleic acid encodes *GMT* and wherein said transformed plant comprises tissue with one or both of
5 an increased α -tocopherol level and increased α -tocotrienol level relative to a plant with a similar genetic background but lacking said introduced first nucleic acid molecule and said introduced second nucleic acid molecule.

24. The transformed plant of claim 21, wherein said transformed plant produces a seed with one or both of an increased γ -tocopherol level and increased γ -tocotrienol level
10 relative to a plant with a similar genetic background but lacking said introduced first nucleic acid molecule and said introduced second nucleic acid molecule.

25. The transformed plant of claim 21, wherein at least one of said introduced first nucleic acid molecule and said introduced second nucleic acid molecule further comprises, in the 5' to 3' direction, an operably linked heterologous promoter.

15 26. The transformed plant of claim 25, wherein said promoter is a seed specific promoter.

27. A transformed plant comprising an introduced first nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, 33 through 38, and an introduced
20 second nucleic acid molecule that encodes an enzyme selected from the group consisting of *tyrA*, *slr1736*, *HPT*, *tocopherol cyclase*, *dxs*, *dxr*, *GGPPS*, *GMT*, *HPPD*, *AANT1*, *slr 1737*, *IDI*, *GGH*, and complements thereof.

28. The transformed plant of claim 27, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica*
25 *napus*, oilseed rape, broccoli, cabbage, citrus, canola, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

29. The transformed plant of claim 27, wherein said introduced second nucleic acid molecule encodes *GMT* and wherein said transformed plant comprises tissue with one or both of an increased α -tocopherol level and increased α -tocotrienol level relative to a plant with a similar genetic background but lacking said introduced first nucleic acid molecule and said introduced second nucleic acid molecule.

30. The transformed plant of claim 27, wherein said transformed plant produces a seed with one or both of an increased γ -tocopherol level and increased γ -tocotrienol level relative to a plant with a similar genetic background but lacking said introduced first nucleic acid molecule and said introduced second nucleic acid molecule.

31. The transformed plant of claim 27, wherein at least one of said introduced first nucleic acid molecule and said introduced second nucleic acid molecule further comprises, in the 5' to 3' direction, an operably linked heterologous promoter.

32. The transformed plant of claim 31, wherein said promoter is a seed specific promoter.

33. A transformed plant comprising an introduced first nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof, and an introduced second nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof.

34. The transformed plant of claim 33, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, citrus, canola, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, chick peas, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

35. The transformed plant of claim 33, wherein said transformed plant produces a seed with increased α -tocopherol levels relative to a plant with a similar genetic

background but lacking said introduced first nucleic acid molecule and said introduced second nucleic acid molecule.

36. The transformed plant of claim 33, wherein at least one of said introduced first nucleic acid molecule and said introduced second nucleic acid molecule comprises, in the 5' to 3' direction, an operably linked heterologous promoter.

37. The transformed plant of claim 36, wherein said promoter is a seed specific promoter.

38. A transformed plant comprising an introduced first nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, 33 through 38, and an introduced second nucleic acid molecule having a sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof.

39. The transformed plant of claim 38, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, citrus, canola, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

40. The transformed plant of claim 38, wherein said transformed plant produces a seed with one or both of an increased α -tocopherol level and increased α -tocotrienol level relative to a plant with a similar genetic background but lacking said introduced first nucleic acid molecule and said introduced second nucleic acid molecule.

41. The transformed plant of claim 38, wherein at least one nucleic acid molecule further comprises, in the 5' to 3' direction, an operably linked heterologous promoter.

42. The transformed plant of claim 41, wherein said promoter is a seed specific promoter.

43. A method for reducing, in a plant, expression of a gene encoding a plant polypeptide having 2-methylphytylplastoquinol methyltransferase activity, comprising:
(A) transforming a plant with a nucleic acid molecule, said nucleic acid molecule having an introduced promoter region which functions in plant cells to cause the production of an mRNA molecule, wherein said introduced promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand, wherein said transcribed strand is complementary to a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 through 15, and wherein said transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the plant cells to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA sequence; and (B) growing said transformed plant.

44. A transformed plant comprising a nucleic acid molecule comprising an introduced promoter region which functions in plant cells to cause the production of an mRNA molecule, wherein said introduced promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand, wherein said transcribed strand is complementary to a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 through 14, and wherein said transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the plant cells to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA sequence.

45. The transformed plant of claim 44, wherein the expression of a gene encoding a plant polypeptide having 2-methylphytylplastoquinol methyltransferase activity is reduced relative to a plant with a similar genetic background but lacking said introduced nucleic acid molecule.

46. A method of producing a plant having a seed with an increased γ -tocopherol level comprising: (A) transforming said plant with an introduced nucleic acid molecule, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the

group consisting of SEQ ID NOs: 1, 2, and 8 through 15; and (B) growing said transformed plant.

47. The method of producing a plant of claim 46, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*,
5 *Brassica napus*, oilseed rape, broccoli, cabbage, canola, citrus, cotton, garlic, oat, *Allium*,
flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry,
sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils,
grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard,
castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

10 48. A method of producing a plant having a seed with one or both of an
increased γ -tocopherol level and increased γ -tocotrienol level comprising: (A) transforming
said plant with an introduced nucleic acid molecule, wherein said nucleic acid molecule
comprises a nucleic acid sequence encoding a polypeptide molecule comprising an amino
acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, and
15 33 through 38; and (B) growing said transformed plant.

49. The method of producing a plant of claim 48, wherein said plant is selected
from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*,
Brassica napus, oilseed rape, broccoli, cabbage, canola, citrus, cotton, garlic, oat, *Allium*,
flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry,
20 sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils,
grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard,
castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

50. A method of producing a plant having a seed with one or both of an
increased γ -tocopherol level and increased γ -tocotrienol level comprising: (A) transforming
25 said plant with an introduced first nucleic acid molecule, wherein said first nucleic acid
molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID
NOs: 1, 2, 8 through 15, and an introduced second nucleic acid molecule encoding an
enzyme selected from the group consisting of *tyrA*, *slr1736*, *HPT*, *tocopherol cyclase*, *dxs*,
dxr, *GGPPS*, *HPPD*, *AANT1*, *slr 1737*, *IDI*, *GGH*, complements thereof, a plant ortholog,

and an antisense construct for homogentisic acid dioxygenase; and (B) growing said transformed plant.

51. The method of producing a plant of claim 50, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*,
5 *Brassica napus*, oilseed rape, broccoli, cabbage, canola, citrus, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

10 52. A method of producing a plant having a seed with one or both of an increased γ -tocopherol level and increased γ -tocotrienol level comprising: (A) transforming said plant with an introduced first nucleic acid molecule, wherein said first nucleic acid molecule comprises a nucleic acid sequence encoding a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through
15 28, 33 through 38, and an introduced second nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA*, *slr1736*, *HPT*, *tocopherol cyclase*, *dxs*, *dxr*, *GGPPS*, *HPPD*, *AANT1*, *slr 1737*, *IDI*, *GGH*, complements thereof, a plant ortholog, and an antisense construct for homogentisic acid dioxygenase; and (B) growing said transformed plant.

20 53. The method of producing a plant of claim 52, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, canola, citrus, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils,
25 grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

54. A method of producing a plant having a seed with one or both of an increased α -tocopherol level and increased α -tocotrienol level comprising: (A) transforming said plant with an introduced first nucleic acid molecule, wherein said first

nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and an introduced second nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof; and (B) growing said transformed plant.

5 55. The method of producing a plant of claim 54, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, canola, citrus, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, 10 grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

 56. A method of producing a plant having a seed with one or both of an increased α -tocopherol level and increased α -tocotrienol level comprising: (A) transforming said plant with an introduced first nucleic acid molecule, wherein said first 15 nucleic acid molecule encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, 33 through 38, and an introduced second nucleic acid molecule that encodes a polypeptide sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof; and (B) growing said transformed plant.

20 57. The method of producing a plant of claim 56, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, canola, citrus, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, 25 grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

 58. Seed derived from a transformed plant, wherein said transformed plant comprises an introduced nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof.

59. The seed of claim 58, wherein said seed has one or both of an increased γ -tocopherol level and increased γ -tocotrienol level relative to a seed from a plant having a similar genetic background but lacking said introduced nucleic acid molecule.

60. Seed derived from a transformed plant, wherein said transformed plant
5 comprises an introduced first nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof, and an introduced second nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA*, *slr1736*, *HPT*, *GMT*, *tocopherol cyclase*, *dxs*, *dxr*, *GGPPS*, *HPPD*, *AANT1*, *slr 1737*, *IDI*, *GGH*, complements thereof, a plant ortholog thereof and an
10 antisense construct for homogentisic acid dioxygenase.

61. The seed of claim 60, wherein said seed has one or both of an increased γ -tocopherol level and increased γ -tocotrienol level relative to a seed from a plant having a similar genetic background but lacking said introduced first nucleic acid molecule and said second nucleic acid molecule.

62. Seed derived from a transformed plant, wherein said transformed plant
15 comprises an introduced first nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof, and an introduced second nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof.

20 63. The seed of claim 62, wherein said seed has one or more of an increased α -tocopherol level and increased α -tocotrienol level relative to a seed from a plant having a similar genetic background but lacking said introduced first nucleic acid molecule and said introduced second nucleic acid molecule.

64. A transformed plant comprising an introduced first nucleic acid molecule
25 comprising a sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof, an introduced second nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof, and an introduced third nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA*, *slr1736*, *HPT*, *tocopherol cyclase*, *dxs*, *dxr*,

GGPPS, *HPPD*, *AANT1*, *slr 1737*, *IDI*, *GGH*, complements thereof, a plant ortholog, and an antisense construct for homogentisic acid dioxygenase.

65. A transformed plant comprising an introduced first nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, 33 through 38, an introduced second nucleic acid molecule having a sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof, and an introduced third nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA*, *slr1736*, *HPT*, *tocopherol cyclase*, *dxs*, *dxr*, *GGPPS*, *HPPD*, *AANT1*, *slr 1737*, *IDI*, *GGH*, complements thereof, a plant ortholog, and an antisense construct for homogentisic acid dioxygenase.

66. A transformed plant comprising an introduced first nucleic acid molecule encoding a tMT2 enzyme and an introduced second nucleic acid molecule encoding a GMT enzyme.

67. A method of producing a plant having seed with an increased α -tocopherol level comprising: (A) transforming said plant with a first nucleic acid molecule encoding a tMT2 enzyme and a second nucleic acid molecule encoding a GMT enzyme; and (B) growing said plant.

68. Oil from the seed of claim 58, 60, or 62.

69. Animal feed comprising the seed of claim 58, 60 or 62.

70. A method of producing a plant having a seed with an increased total tocopherols level comprising: (A) transforming said plant with an introduced nucleic acid molecule, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, and 8 through 15; and (B) growing said transformed plant.

71. The method of producing a plant of claim 70, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, canola, citrus, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils,

grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

Query= Arabidopsis TMT2
(338 letters)

	Score (bits)	E Value
Sequences producing significant alignments:		
Brassical - LIB4153-013-R1-K1-B7	668	0.0
Brassica2 - LIB80-011-Q1-E1-E9	668	0.0
Glycine max TMT2 - LIB3049-032-Q1-E1-G8	569	e-166
Gossypium hirsutum TMT2 -LIB3272-054-P1-K1-C11	564	e-164
Allium Porrum - Lib4521-015-Q1-K1-D6	520	e-151
Zea mays TMT2- LIB3587-273-Q1-K6-C5/LIB3600-046-Q1-K6-G1	508	e-148
Oryza sativa TMT2 - LIB4371-041-R1-K1-F7	506	e-147
>Brassical - LIB4153-013-R1-K1-B7 Length = 337		
Score = 668 bits (1706), Expect = 0.0		
Identities = 322/339 (94%), Positives = 328/339 (95%), Gaps = 3/339 (0%)		
>Brassica2 - LIB80-011-Q1-E1-E9 Length = 337		
Score = 668 bits (1706), Expect = 0.0		
Identities = 322/339 (94%), Positives = 328/339 (95%), Gaps = 3/339 (0%)		
>Glycine max TMT2 - LIB3049-032-Q1-E1-G8 Length = 342		
Score = 569 bits (1450), Expect = e-166		
Identities = 276/346 (79%), Positives = 298/346 (85%), Gaps = 12/346 (3%)		
>Gossypium hirsutum TMT2 -LIB3272-054-P1-K1-C11 Length = 341		
Score = 564 bits (1437), Expect = e-164		
Identities = 274/345 (79%), Positives = 297/345 (85%), Gaps = 11/345 (3%)		
>Allium Porrum - Lib4521-015-Q1-K1-D6 Length = 344		
Score = 520 bits (1325), Expect = e-151		
Identities = 261/352 (74%), Positives = 289/352 (81%), Gaps = 22/352 (6%)		
>Zea mays TMT2- LIB3587-273-Q1-K6-C5/LIB3600-046-Q1-K6-G1 Length = 352		
Score = 508 bits (1293), Expect = e-148		
Identities = 243/329 (73%), Positives = 276/329 (83%), Gaps = 5/329 (1%)		
>Oryza sativa TMT2 - LIB4371-041-R1-K1-F7 Length = 348		
Score = 506 bits (1288), Expect = e-147		
Identities = 247/333 (74%), Positives = 279/333 (83%), Gaps = 7/333 (2%)		

Fig. 2

		1		50	
SEQ ID NO.	22	corn	MAMAST.YAP	GGGARA.LAQ	GRCRVGPAG LGFLGPS.KA AGLPRPLALA
SEQ ID NO.	26	rice	MAMASSAYAP	AGGVGTHSAP	G..RIRPPRG LGF...S.TT TTKSRPLVLT
SEQ ID NO.	27	Brassical	--MASLML..	.NGAITF...PKG LGFPASNHLA R..PSPPLSL
SEQ ID NO.	28	ArabidopsisT	--MASLML..	.NGAITF...PKG LGSPGSNLHA RSI PRPTLLS
SEQ ID NO.	23	Cotton	--MASSML..	.NGAETFT..	.LIRGVTPKS IGFLGSGLHG KQFS..SAGL
SEQ ID NO.	25	soy	--MGSVML..	.SGTEKLT..	.L.RTLTGNG LGFTGSDLHG KNFPRVSFAA
SEQ ID NO.	24	Leek	--MASSML..	.SGAESLS..	.MLRIHHQPK LTFSSPSLHS KPTNLKMDLI
SEQ ID NO.	108	Consensus	--M-S-----	--G-----	-----
		51		100	
SEQ ID NO.	22	corn	LARRMSSPVA	VGARLRCAAS	SSPAAARPAT APRFIQHKKE AFWFYRFLSI
SEQ ID NO.	26	rice	RRGGGGGNIS	V.ARLRCAAS	SSSAAARPMs QPRFIQHKKE AFWFYRFLSI
SEQ ID NO.	27	Brassical	VSNTATRRL.	.SVATRCSSS	SSVSASRPSA QPRFIQHKKE AYWFYRFLSI
SEQ ID NO.	28	ArabidopsisT	VRTSTPRL.	.SVATRC.SS	SSVSSSRPSA QPRFIQHKKE AYWFYRFLSI
SEQ ID NO.	23	Cotton	IYSPKMSRVG	TTIAPRC...	.SLSASRPAS QPRFIQHKKE AFWFYRFLSI
SEQ ID NO.	25	soy	TTSAKVPNFR	SIVVPC...	.SVSASRPSS QPRFIQHKKE AFWFYRFLSI
SEQ ID NO.	24	Leek	PFATKHQKTK	KASIFTCSAS	SS...SRPAS QPRFIQHKQE AFWFYRFLSI
SEQ ID NO.	108	Consensus	-----	-----C---	-S----RP-- -PRFIQHK-E A-WFYRFLSI
		101		150	
SEQ ID NO.	22	corn	VYDHVINPGH	WTEDMRDDAL	EPADLFSRHL TVVDVGGGTG FTTLGIVKHV
SEQ ID NO.	26	rice	VYDHVINPGH	WTEDMRDDAL	EPADLYSRKL RVVDVGGGTG FTTLGIVKRV
SEQ ID NO.	27	Brassical	VYDHIINPGH	WTEDMRDDAL	EPADLSHPDM RVVDVGGGTG FTTLGIVKTV
SEQ ID NO.	28	ArabidopsisT	VYDHVINPGH	WTEDMRDDAL	EPADLSHPDM RVVDVGGGTG FTTLGIVKTV
SEQ ID NO.	23	Cotton	VYDHVINPGH	WTEDMRDDAL	EPADLNDRDM VVVDVGGGTG FTTLGIVQHV
SEQ ID NO.	25	soy	VYDHVINPGH	WTEDMRDDAL	EPADLNDRNM IVVDVGGGTG FTTLGIVKHV
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Fig. 3a

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SEQ ID NO.	28	Arabidopsist	GSIEYWDPDQ	RGIREAYRVL	KIGGKACLIG	PVYPTFWLSR	FFSDVWMLFP
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SEQ ID NO.	27	Brassical	SPLQLGPKKE	DVEKPVNNPF	SFLGRFLLGT	LAAAWFVLIP	IYMWIKDQIV
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Fig. 3b

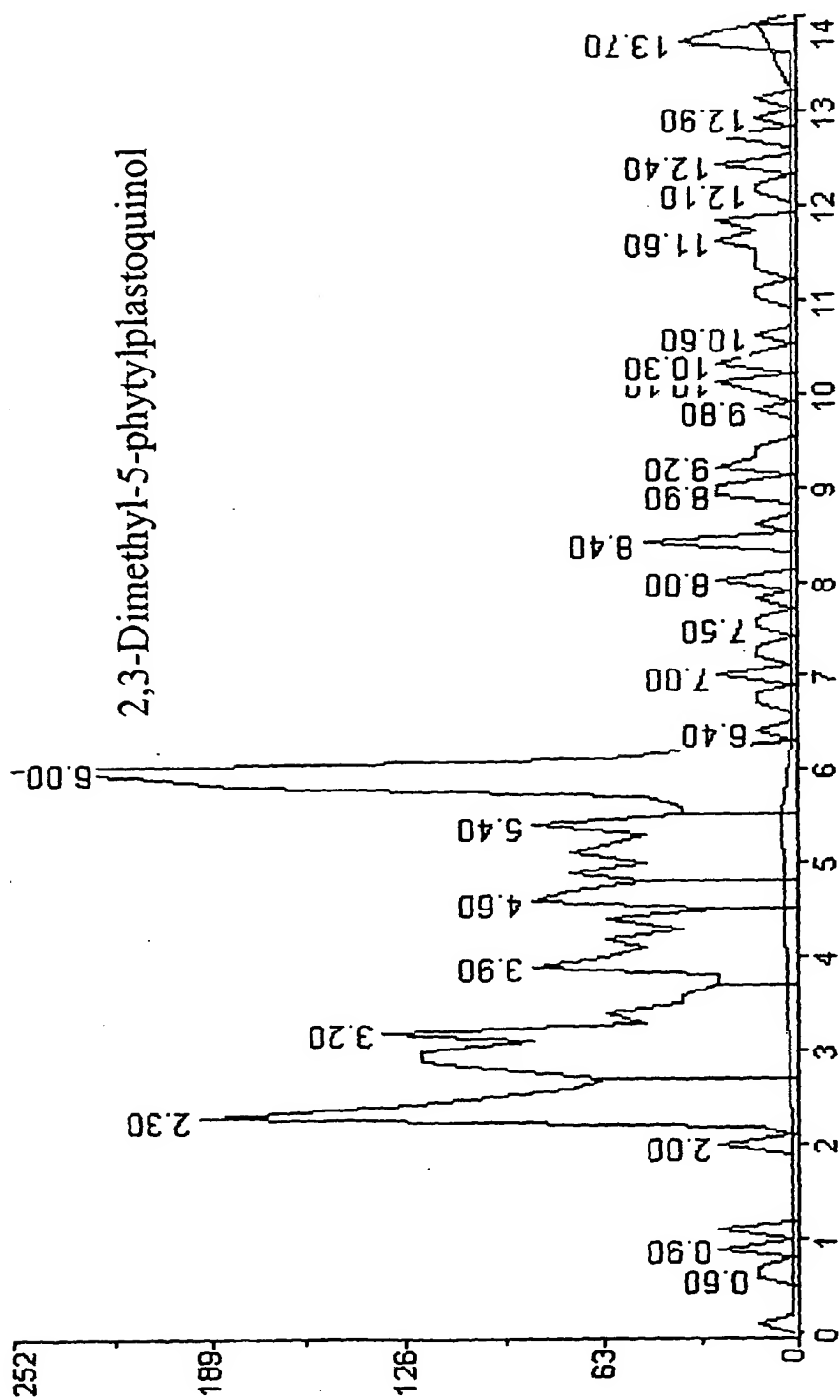


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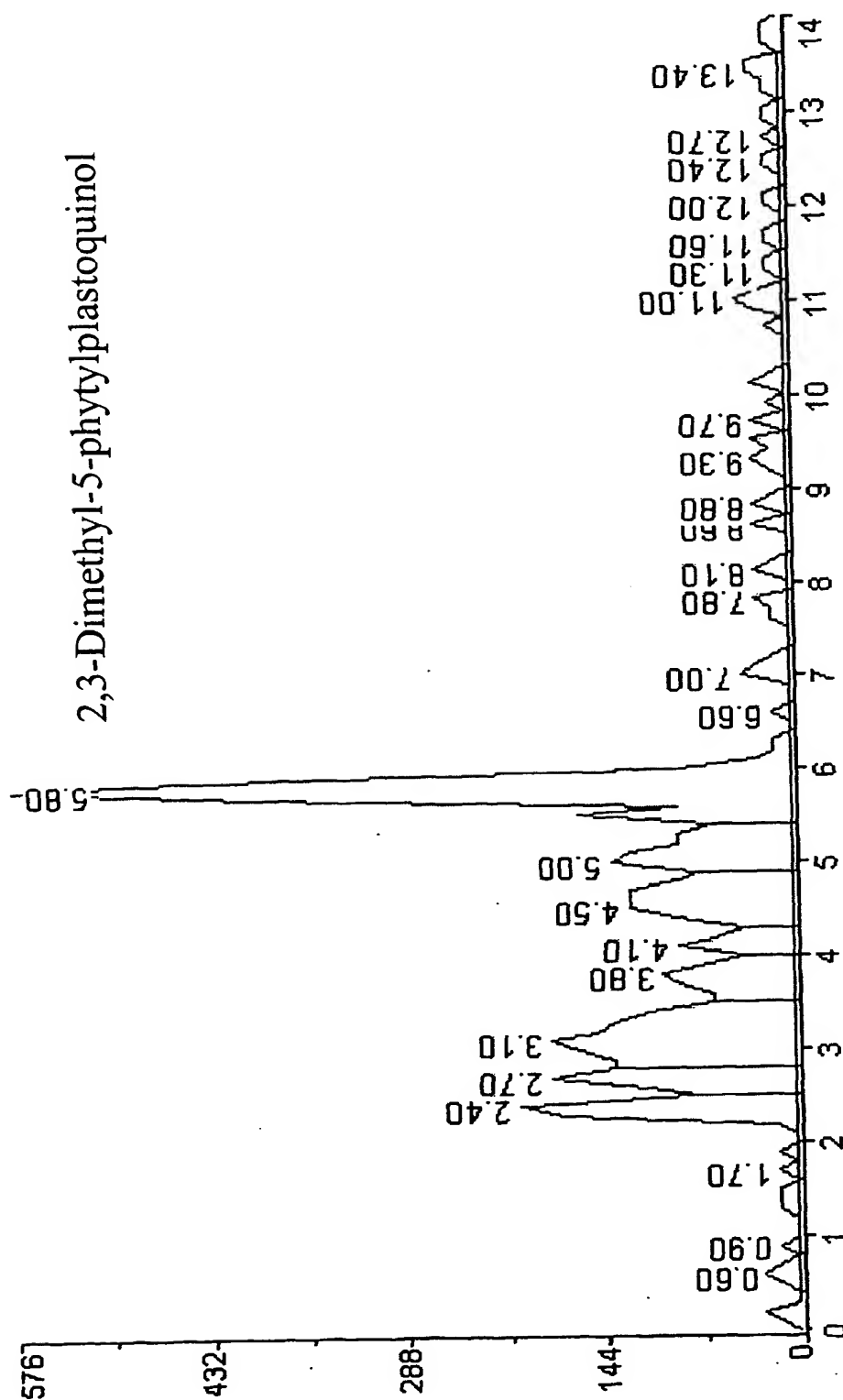


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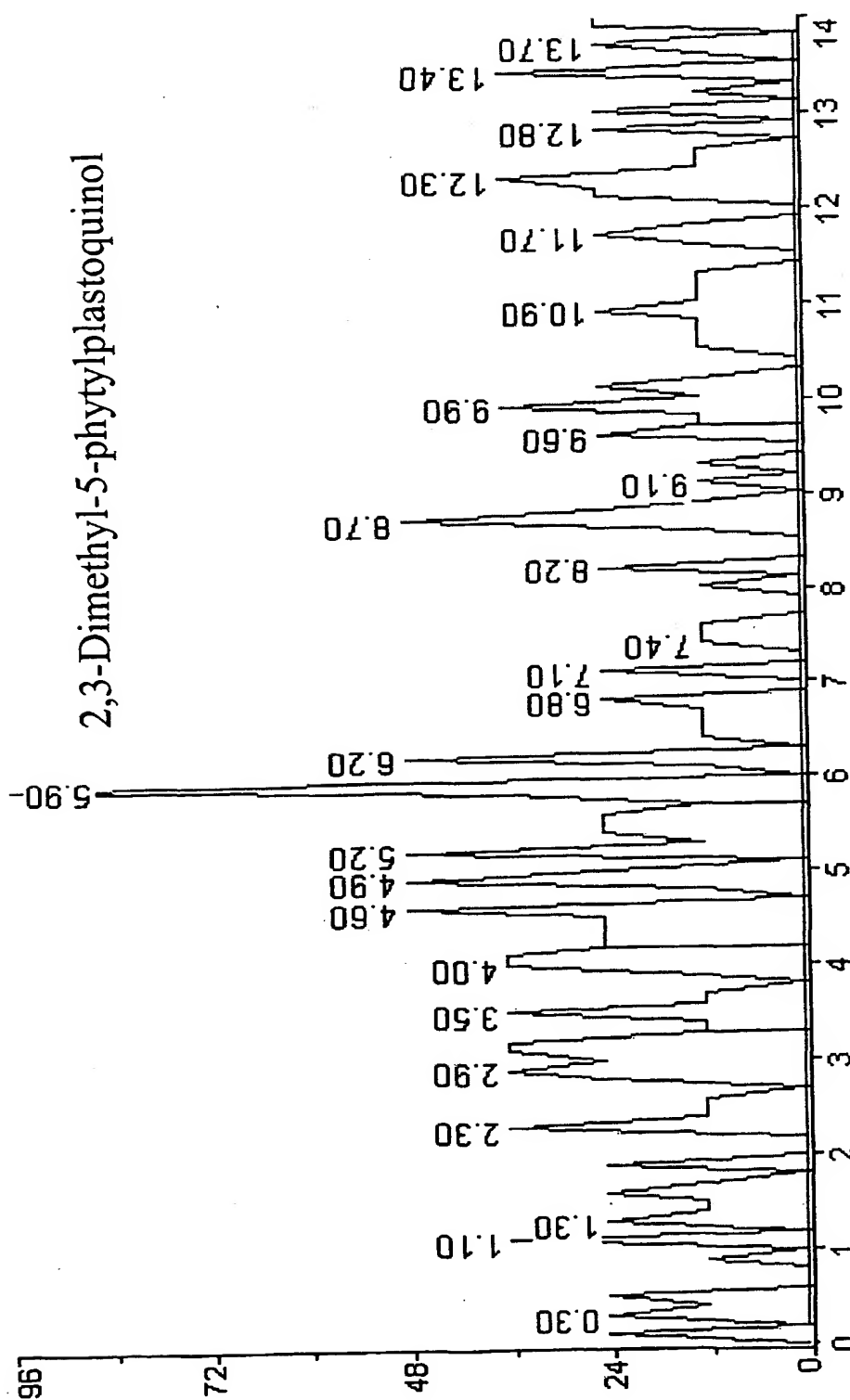


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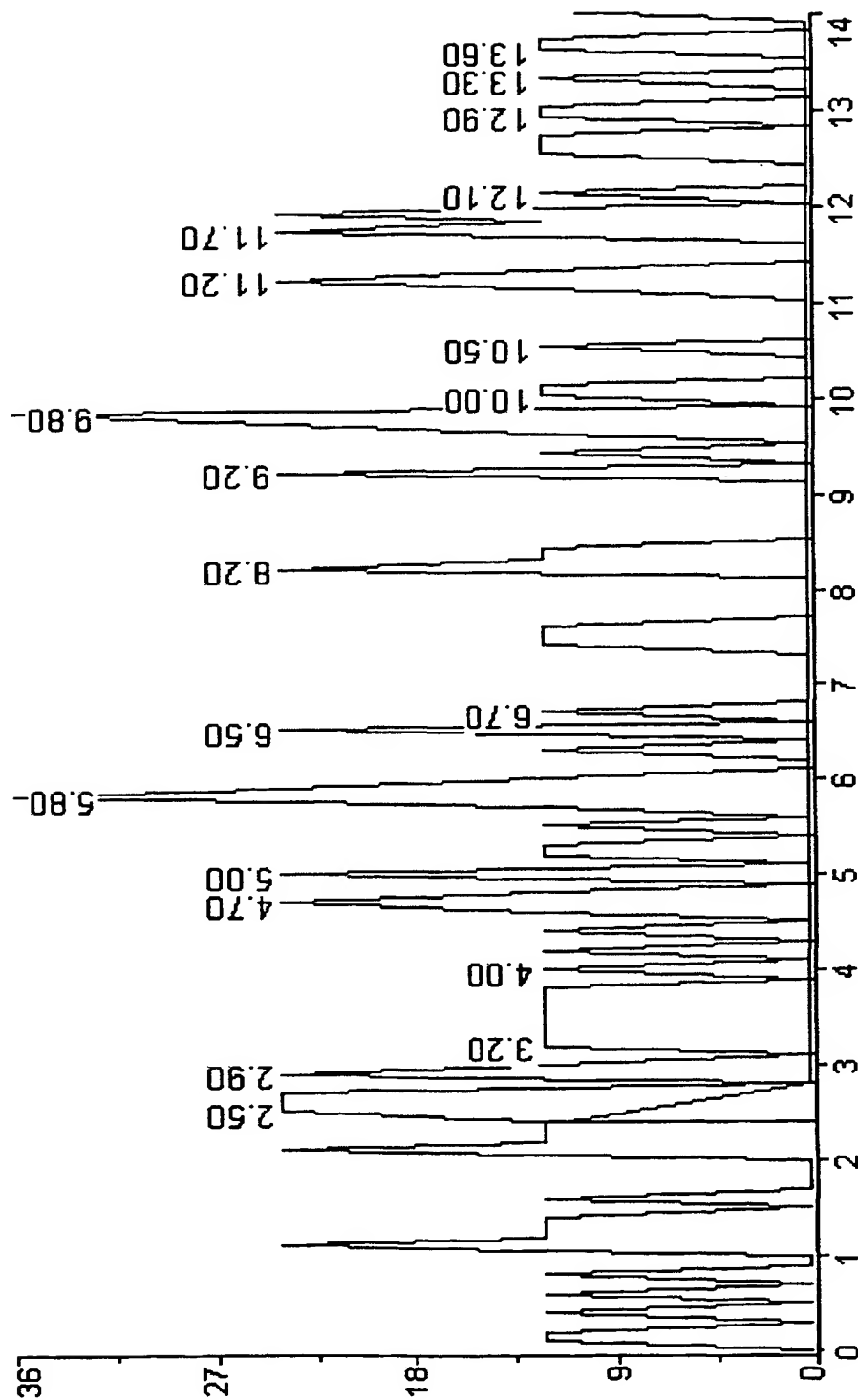


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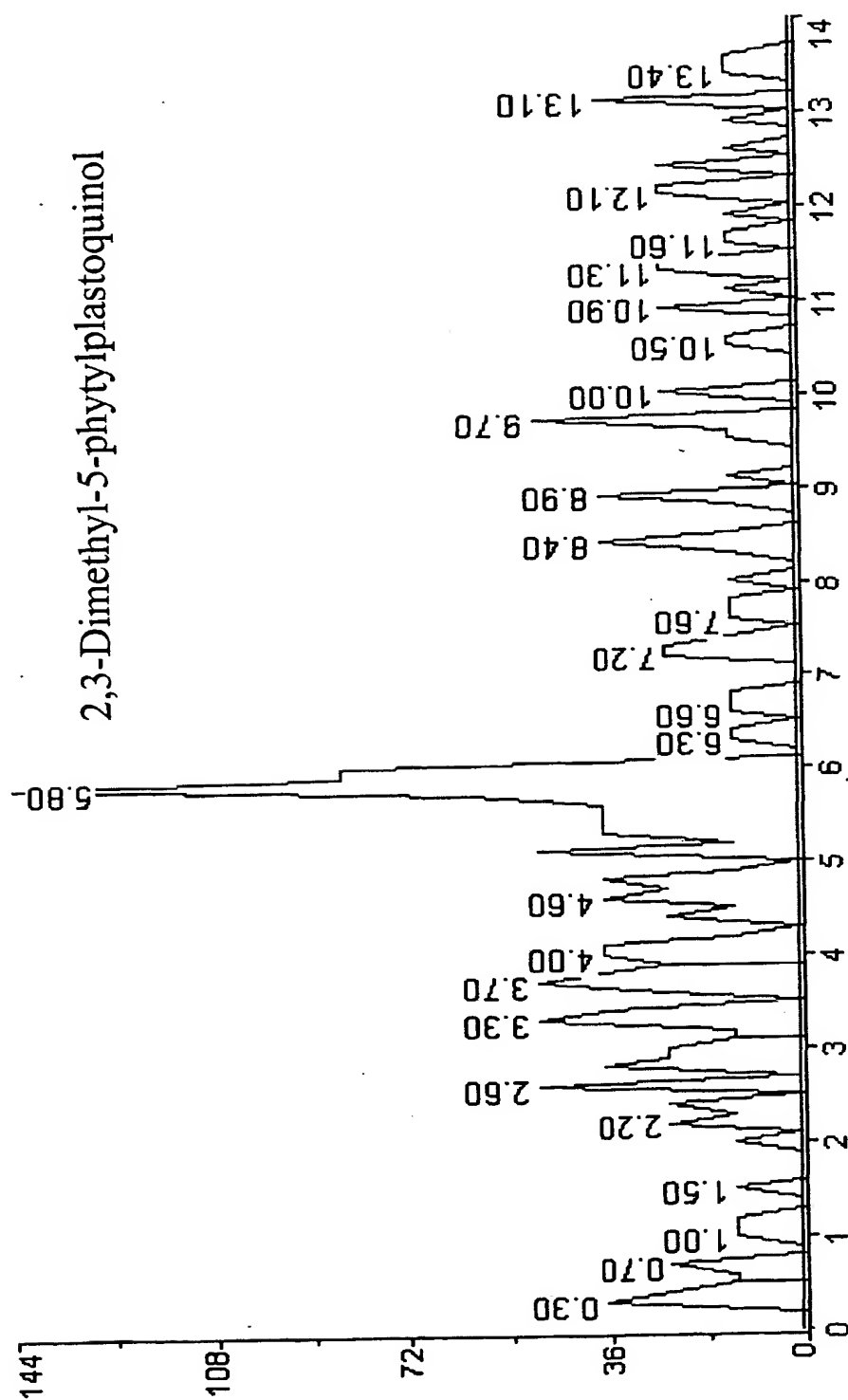
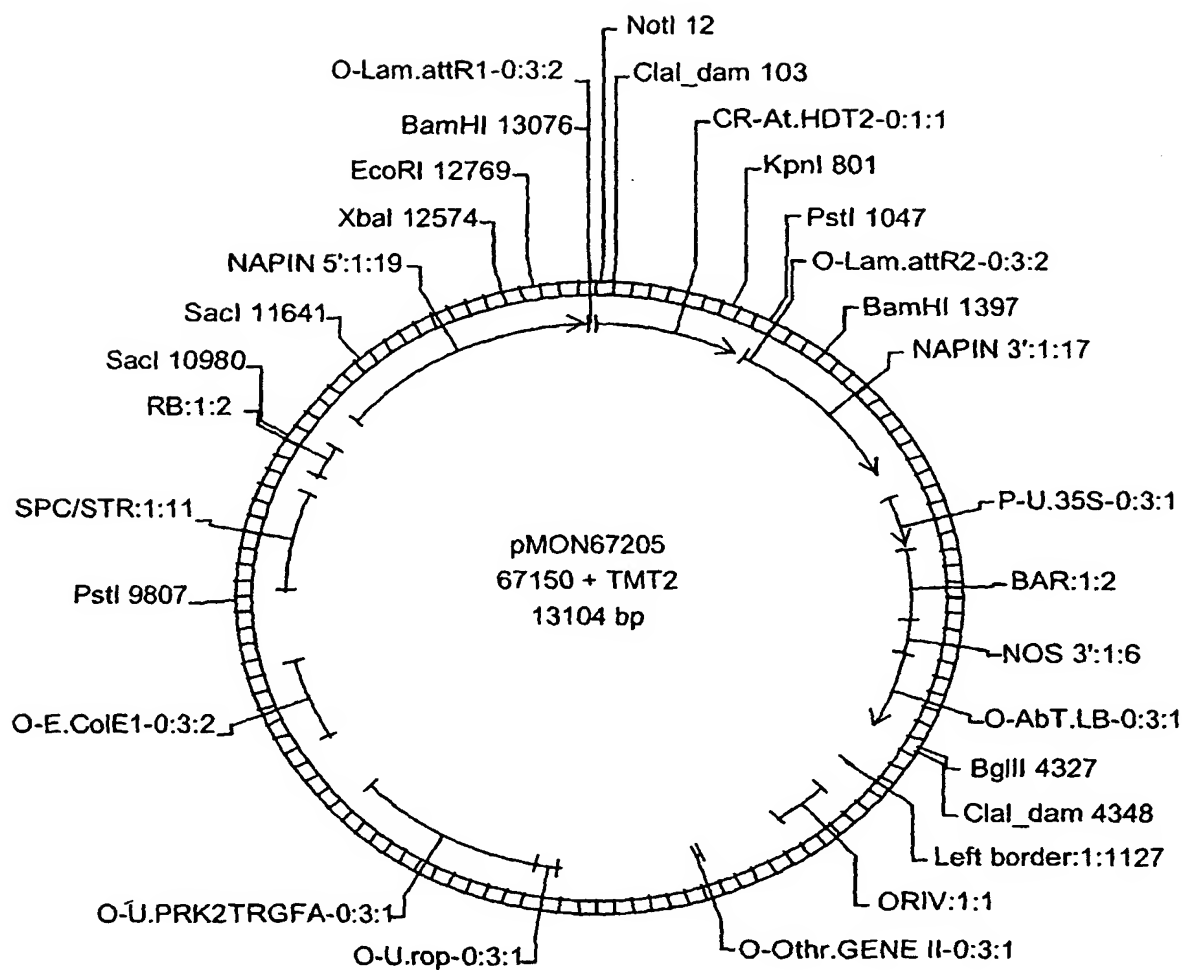
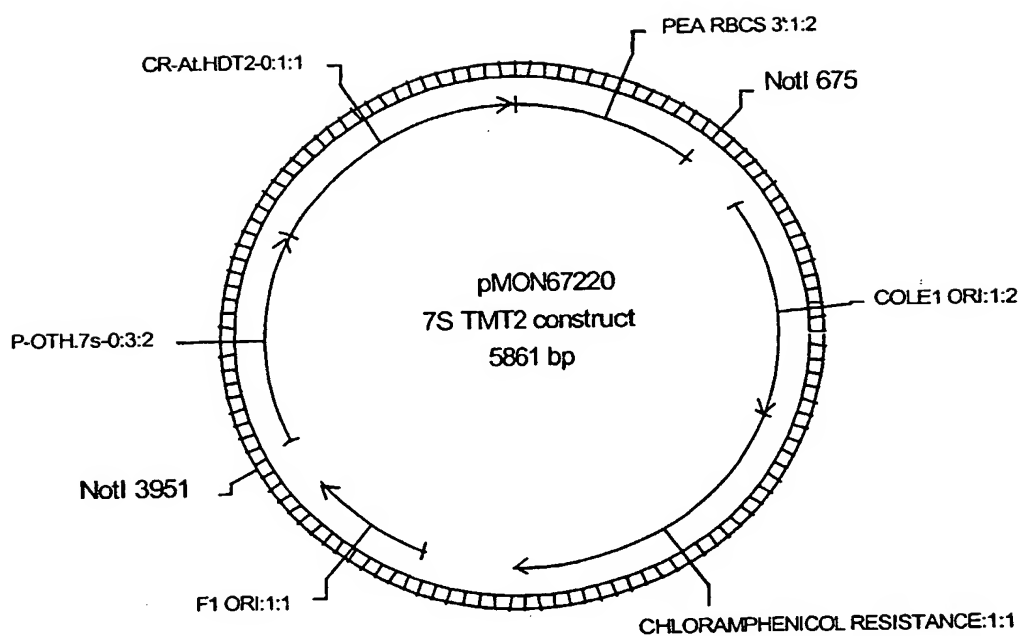
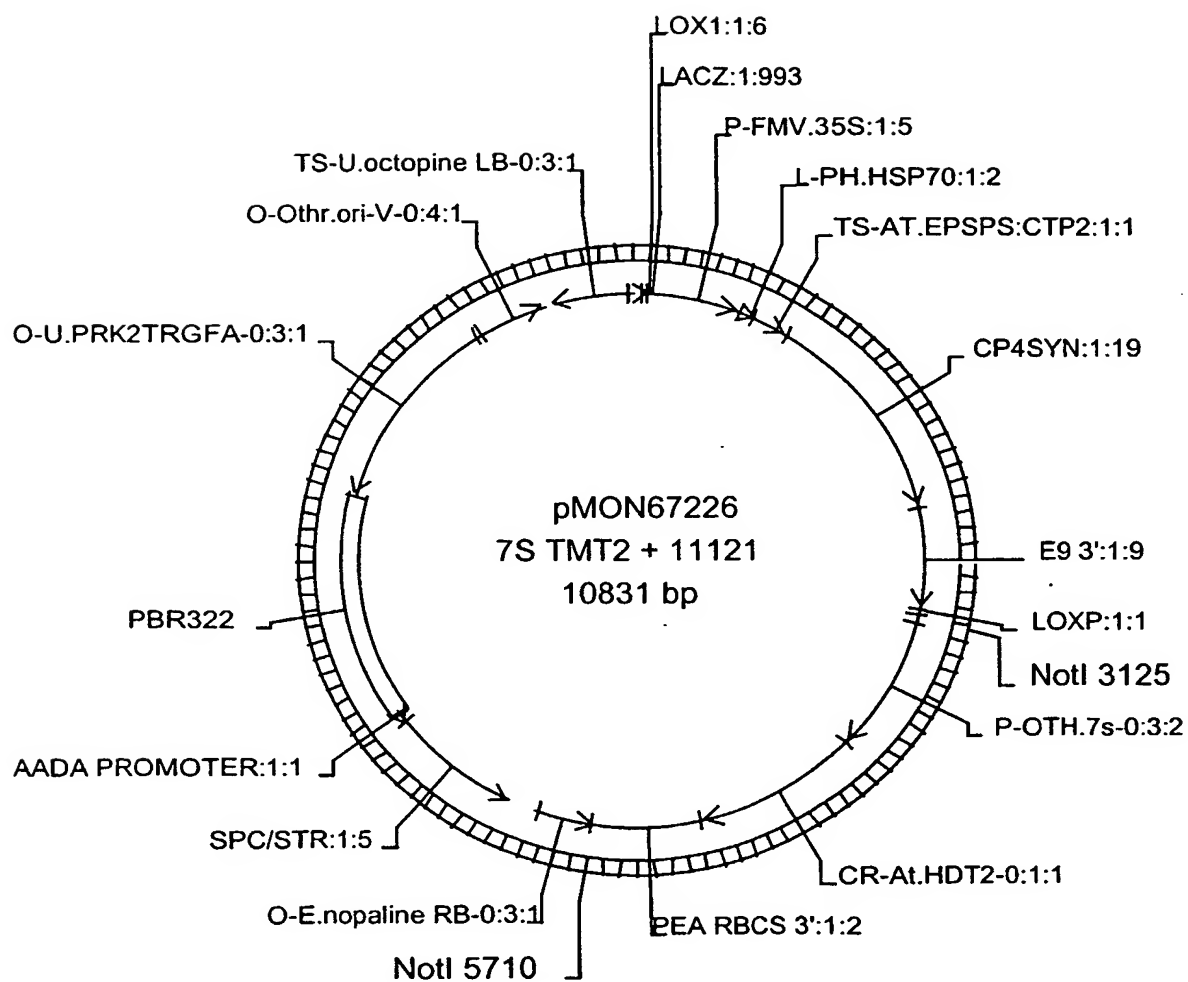
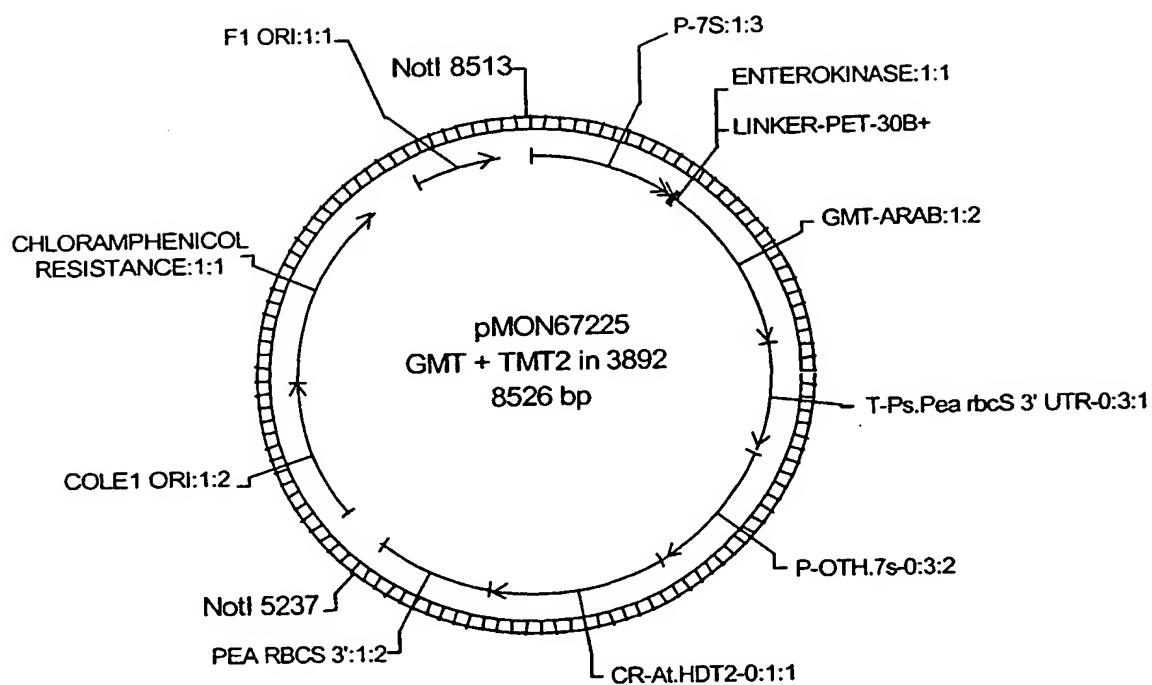


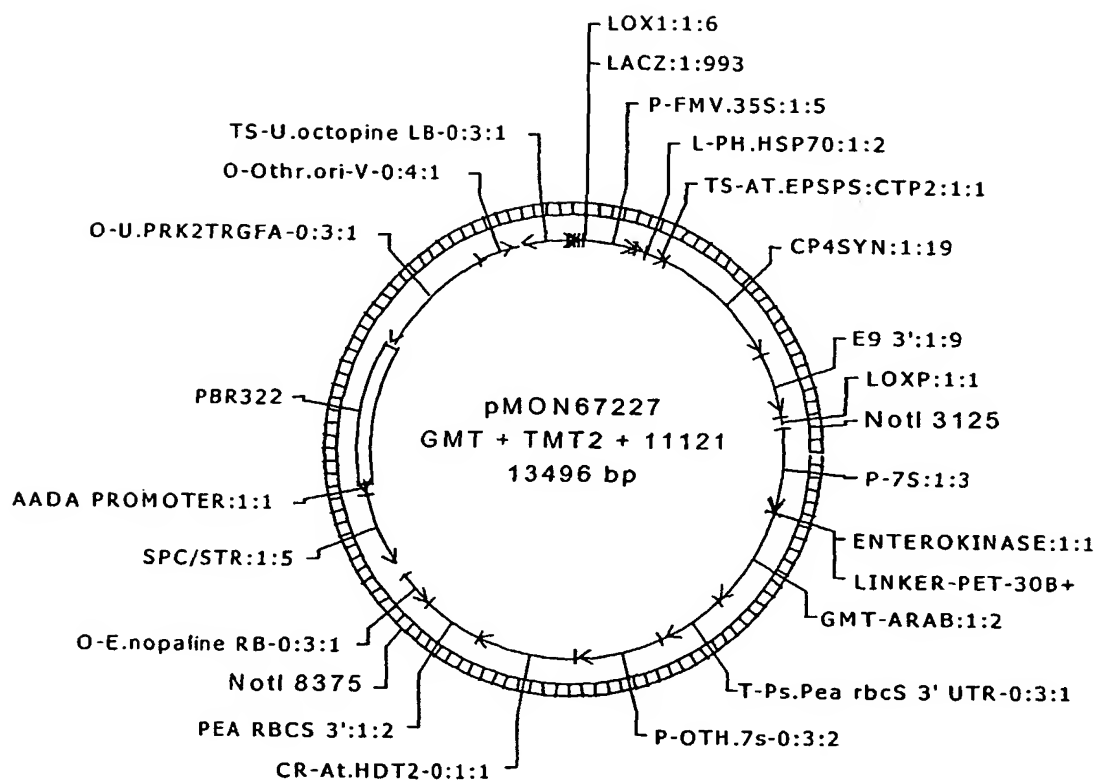
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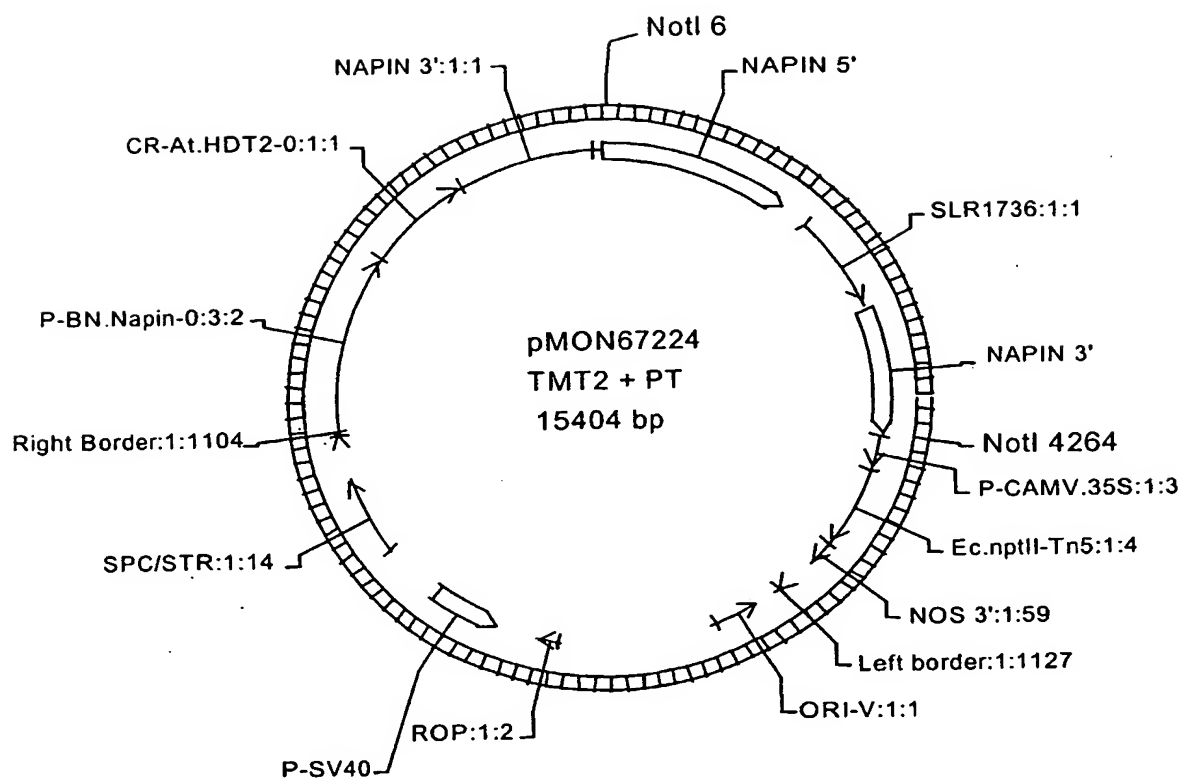
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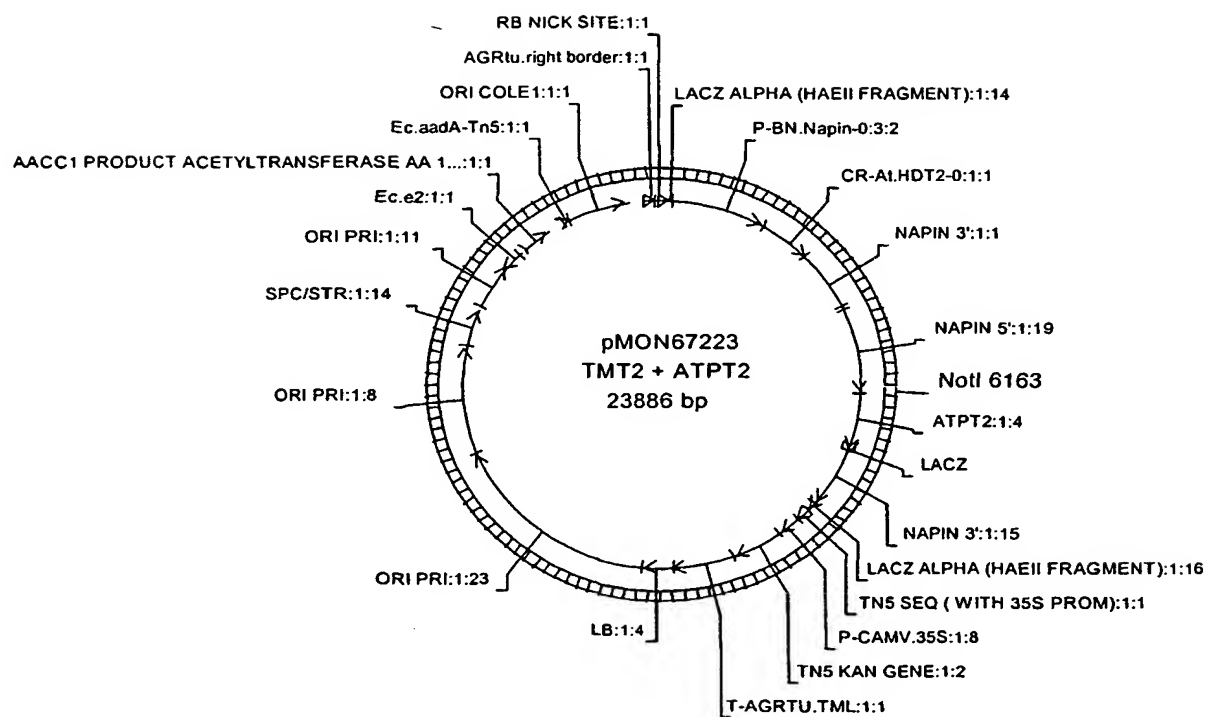
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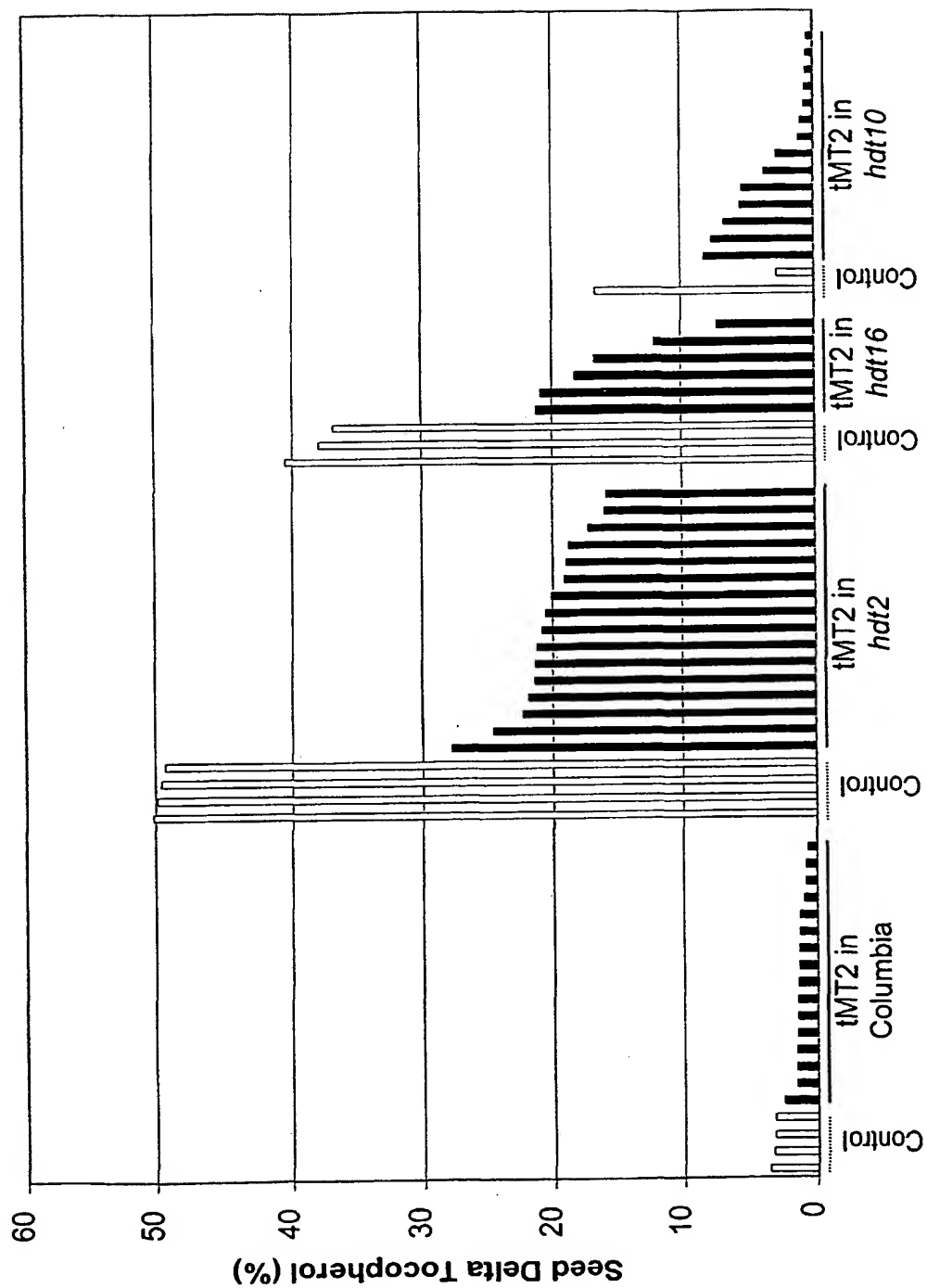
*Fig. 11*

*Fig. 12*

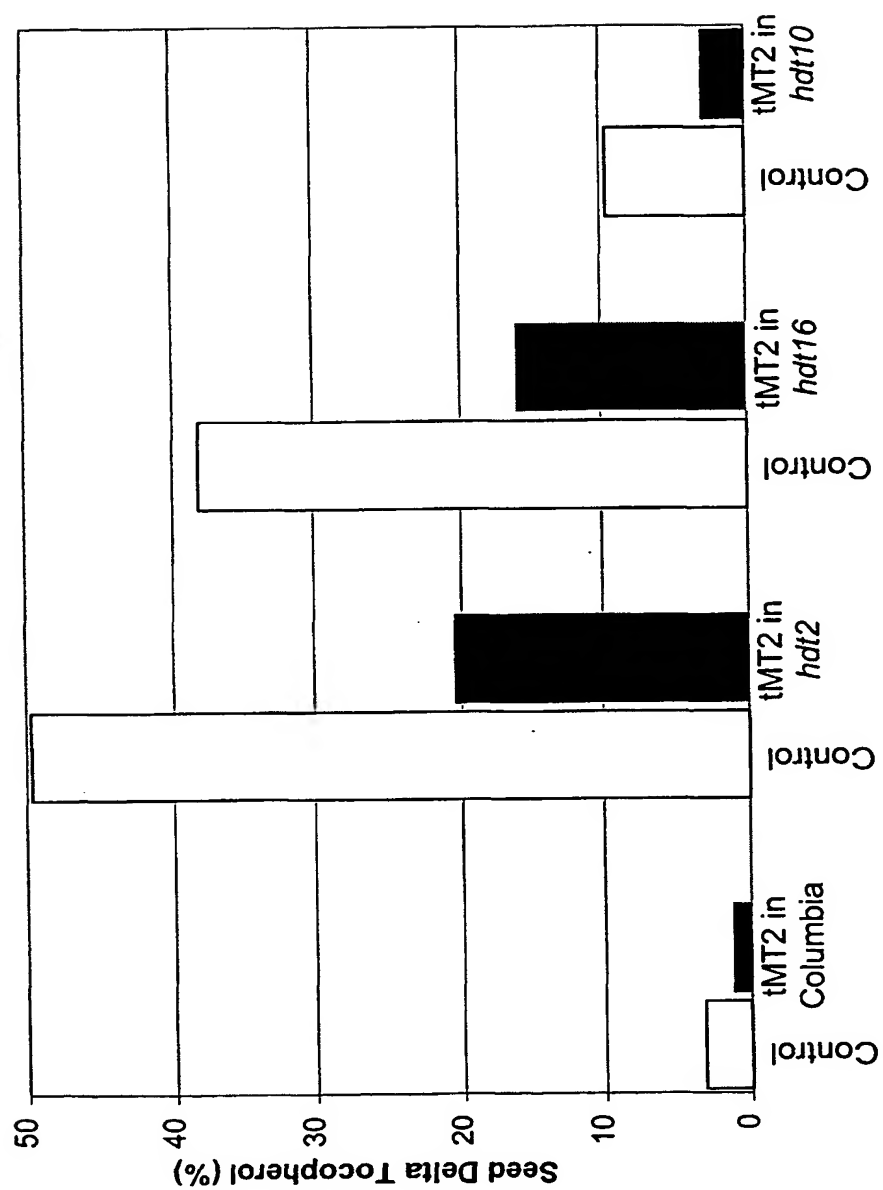
*Fig. 13*

*Fig. 14*

**Fig. 15**



Arabidopsis
Fig. 16a



Arabidopsis

Fig. 16b

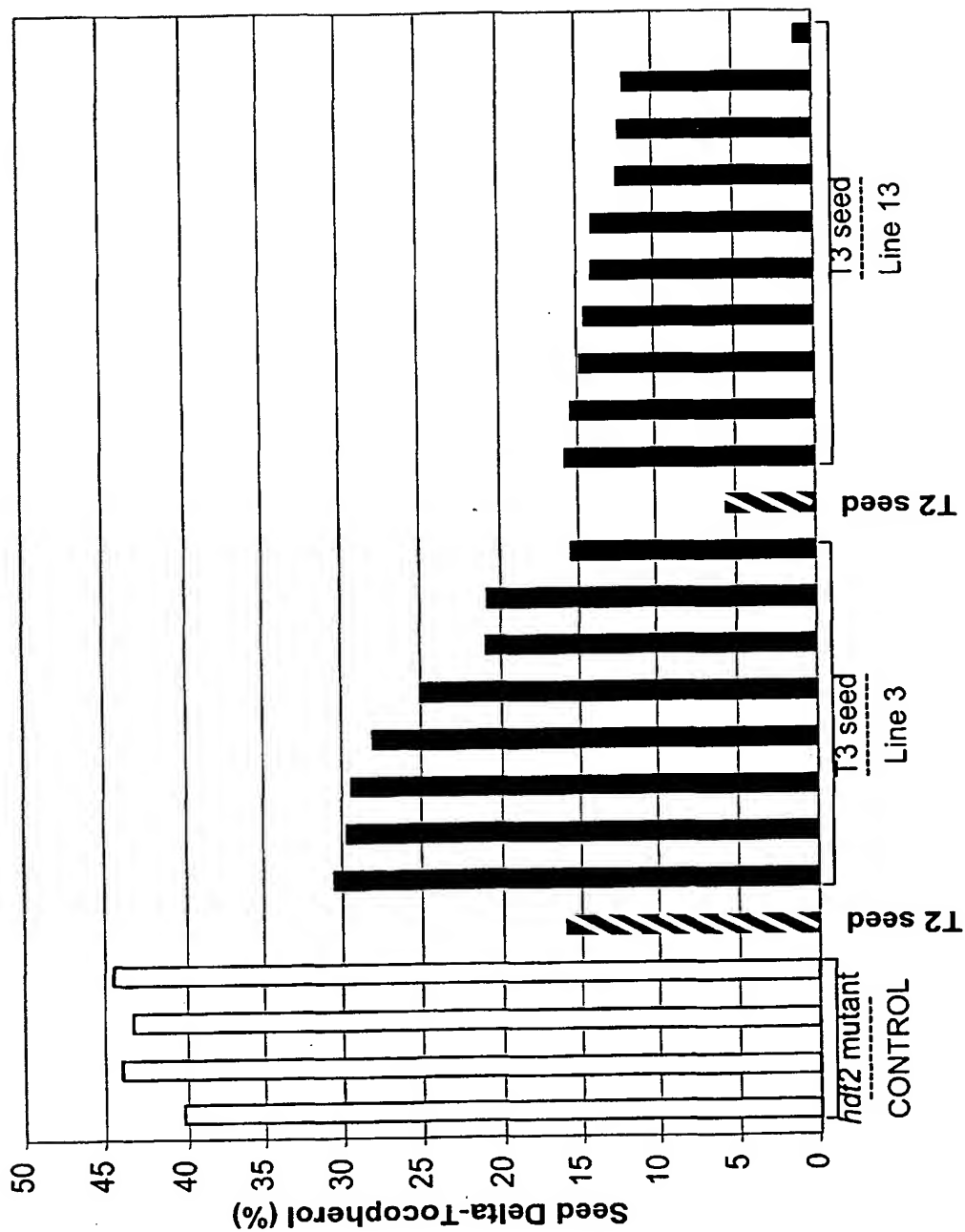
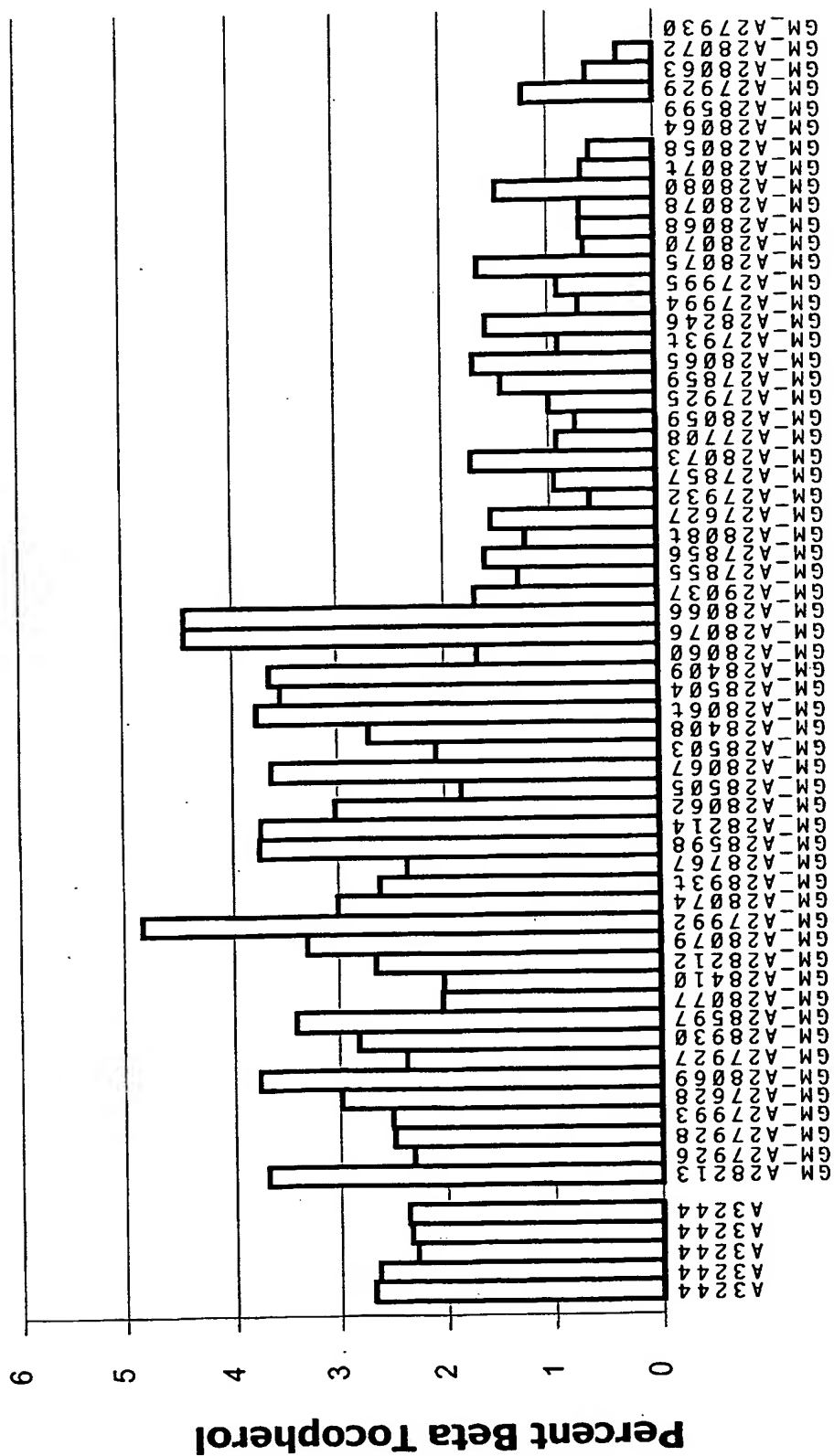


Fig. 17



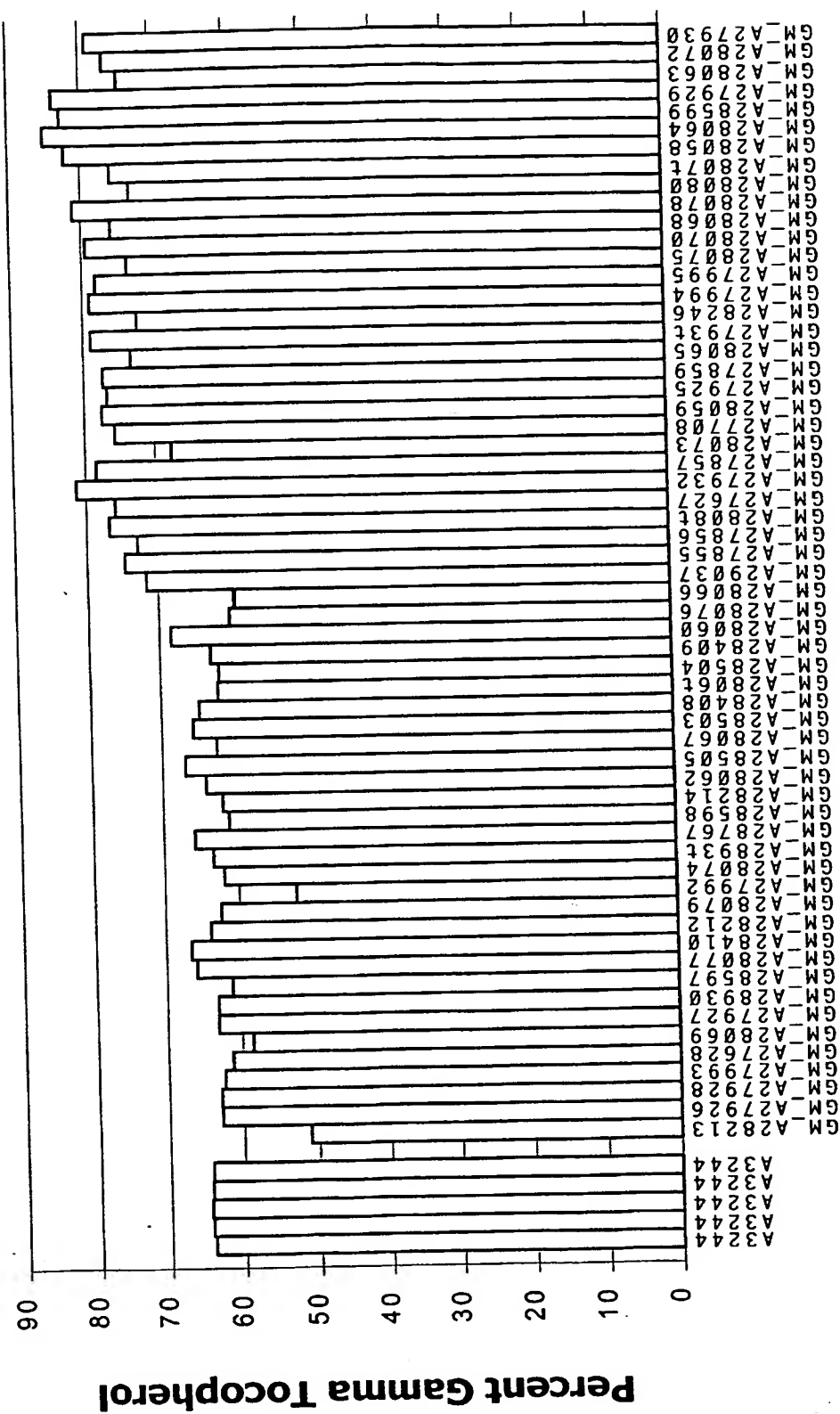


Fig. 18c

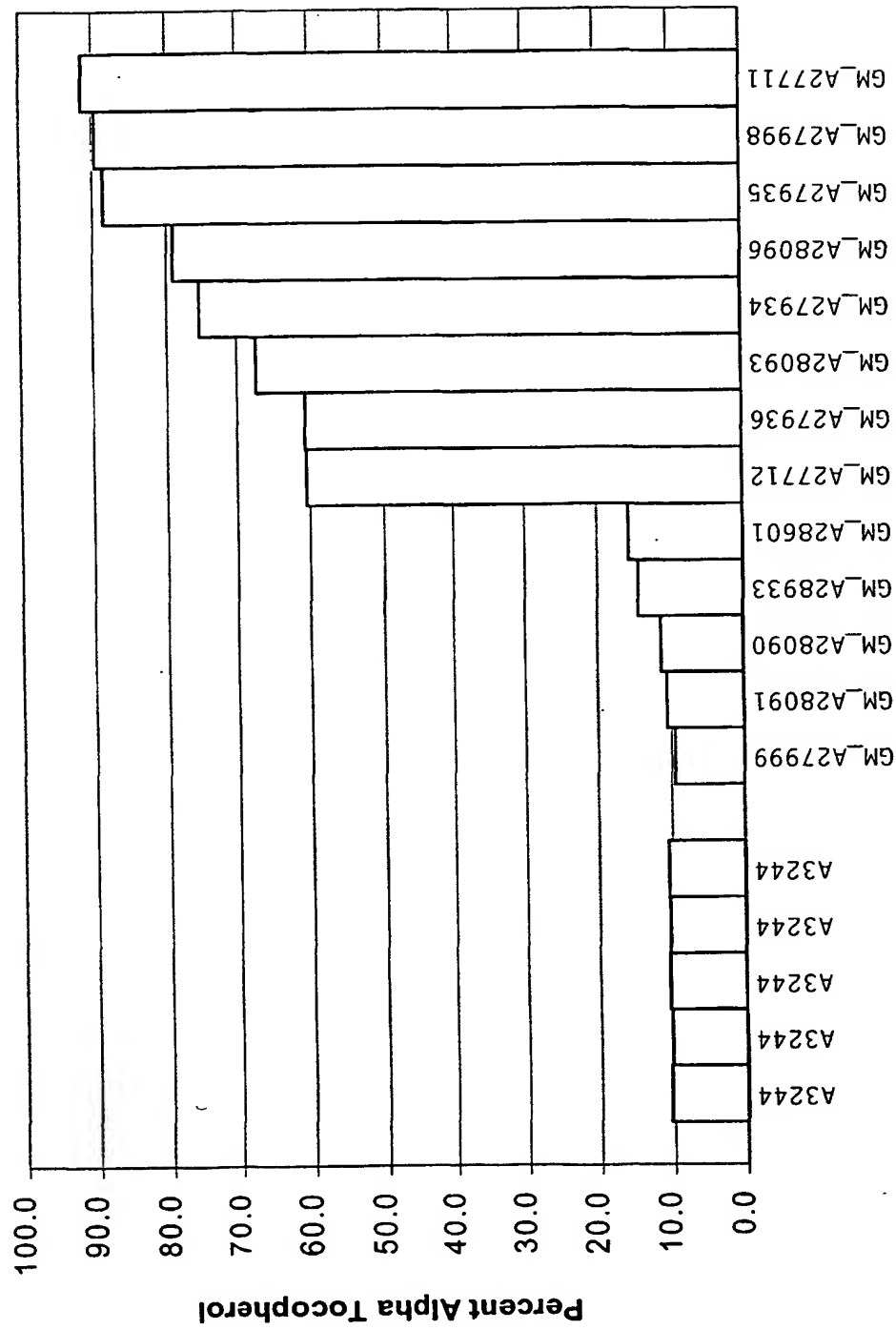


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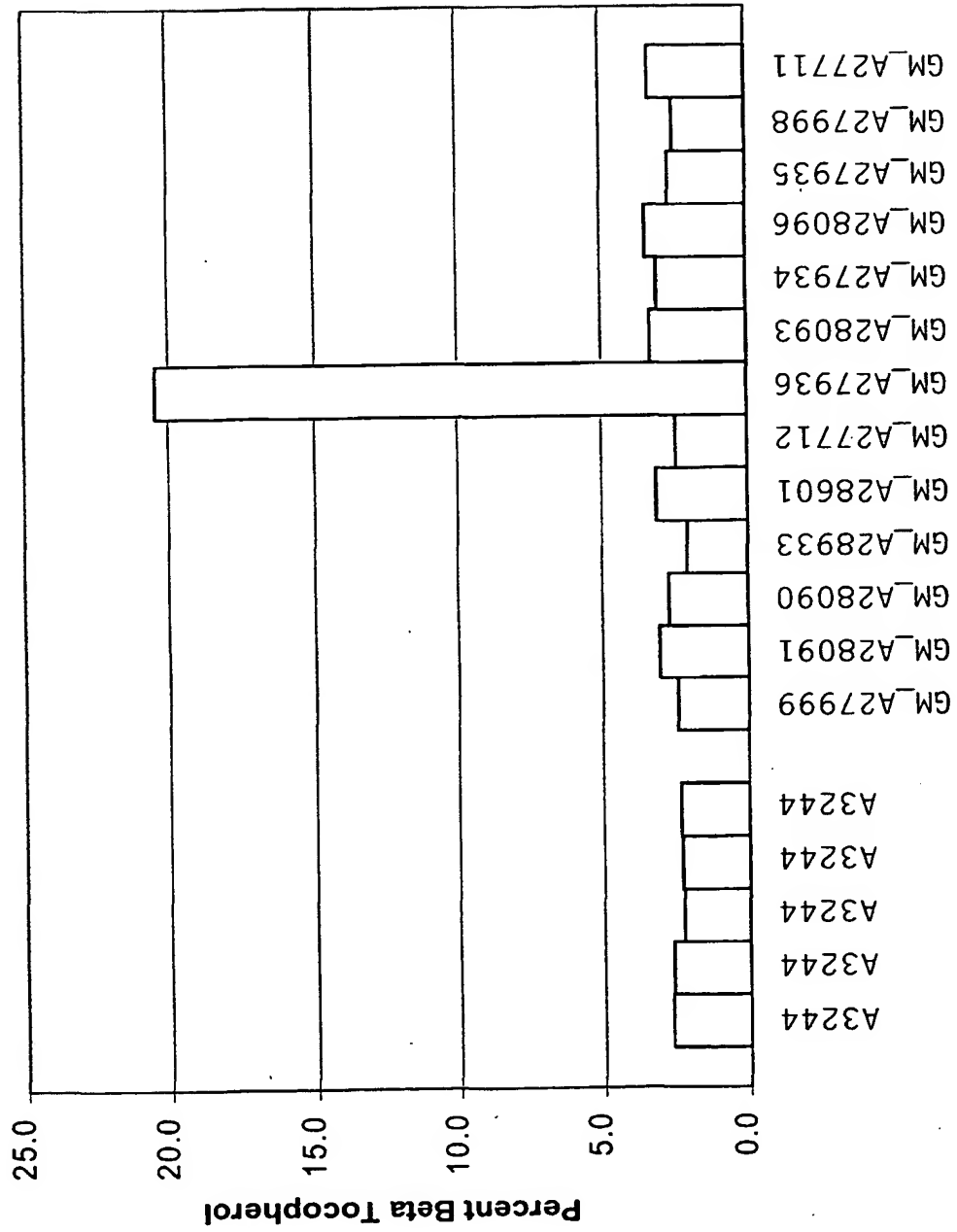
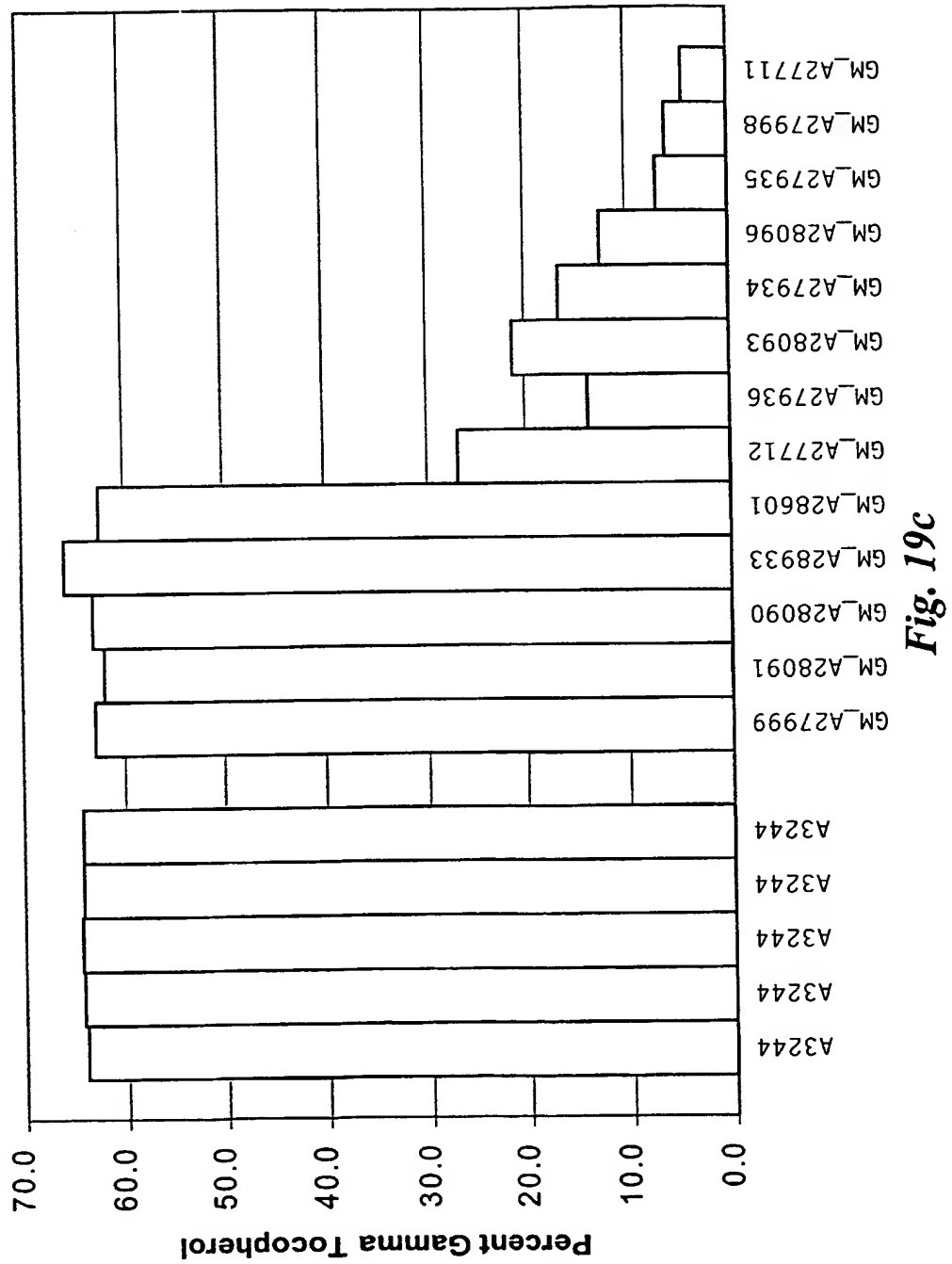


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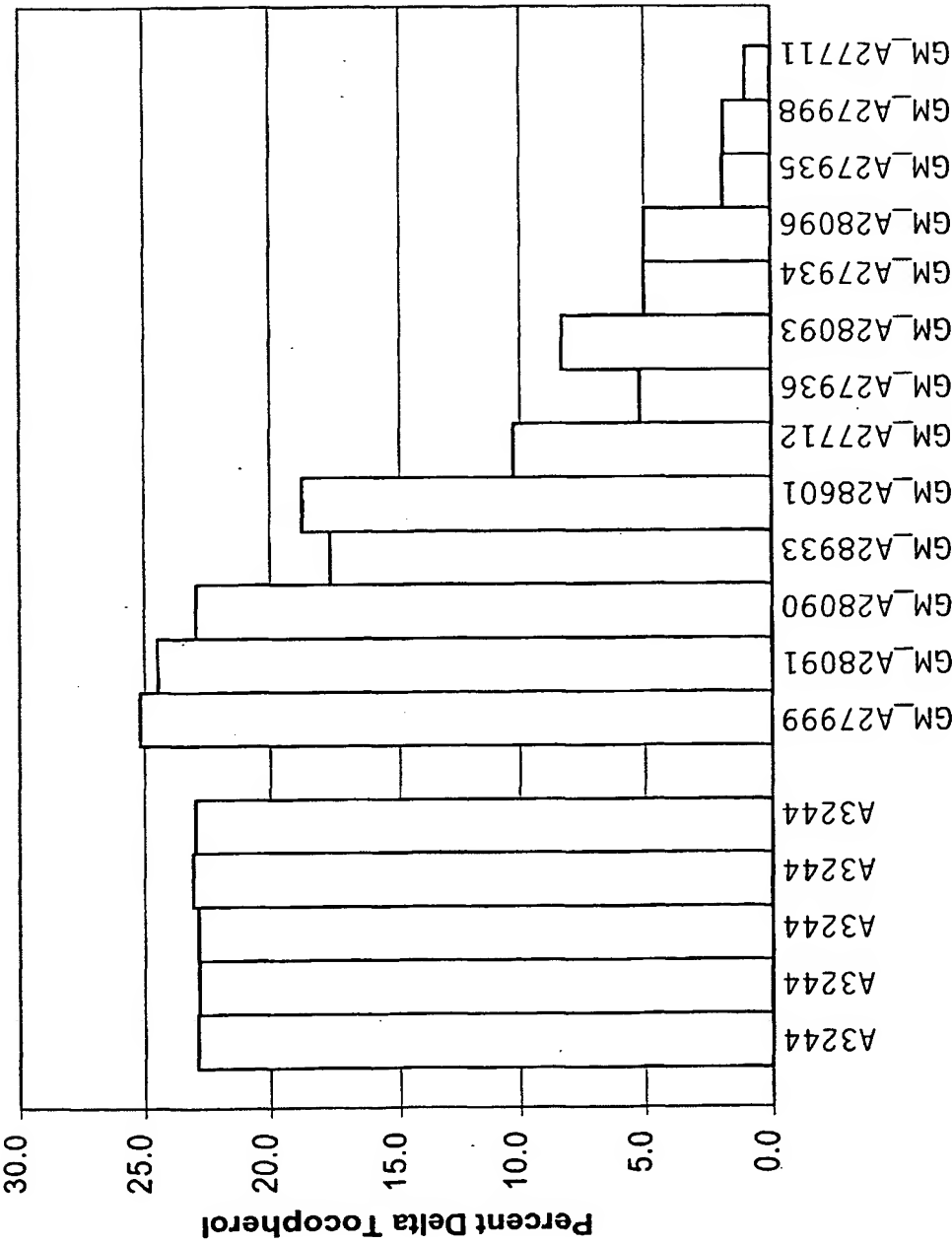


Fig. 19d

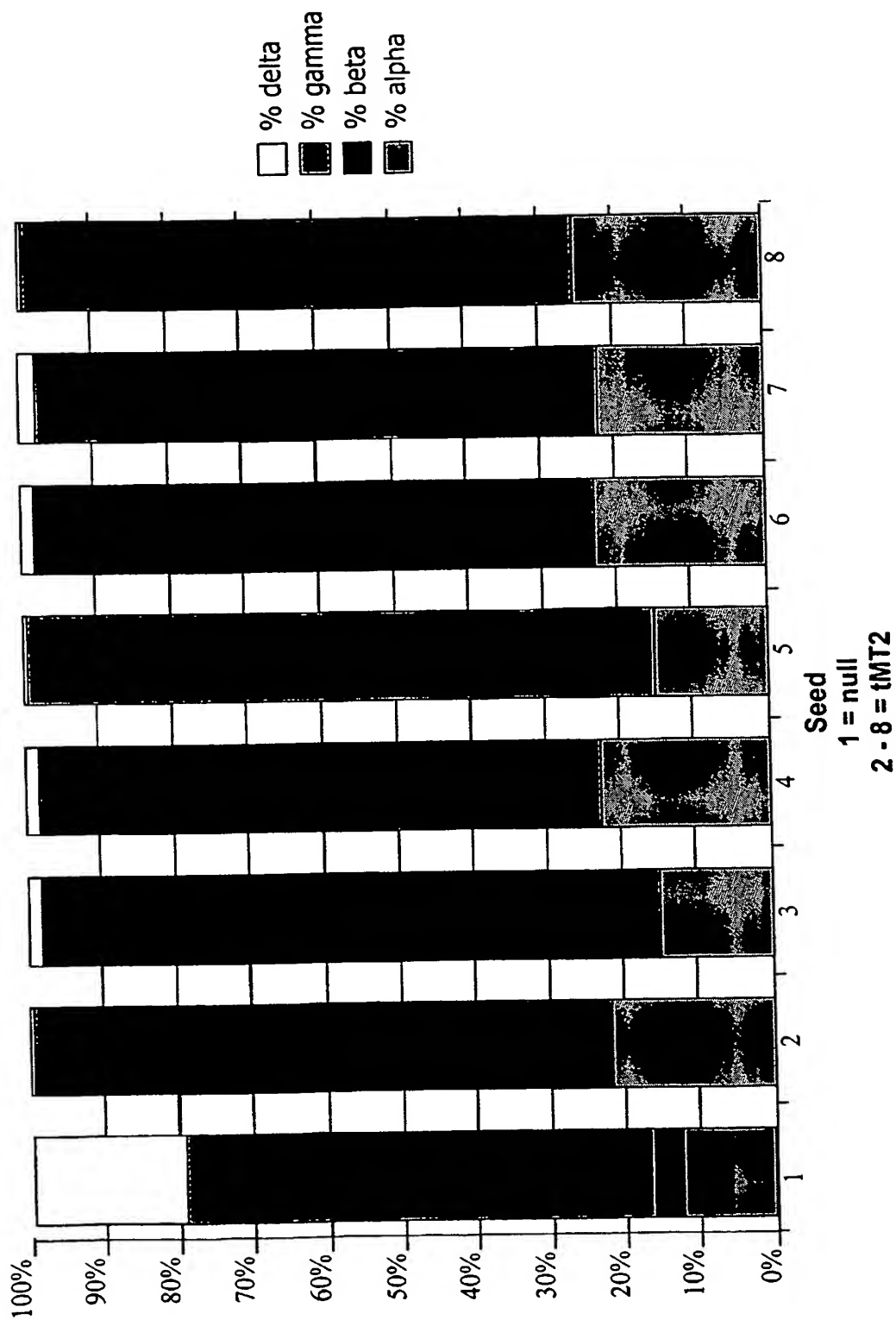
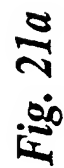
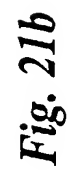
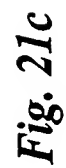
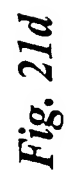


Fig. 20





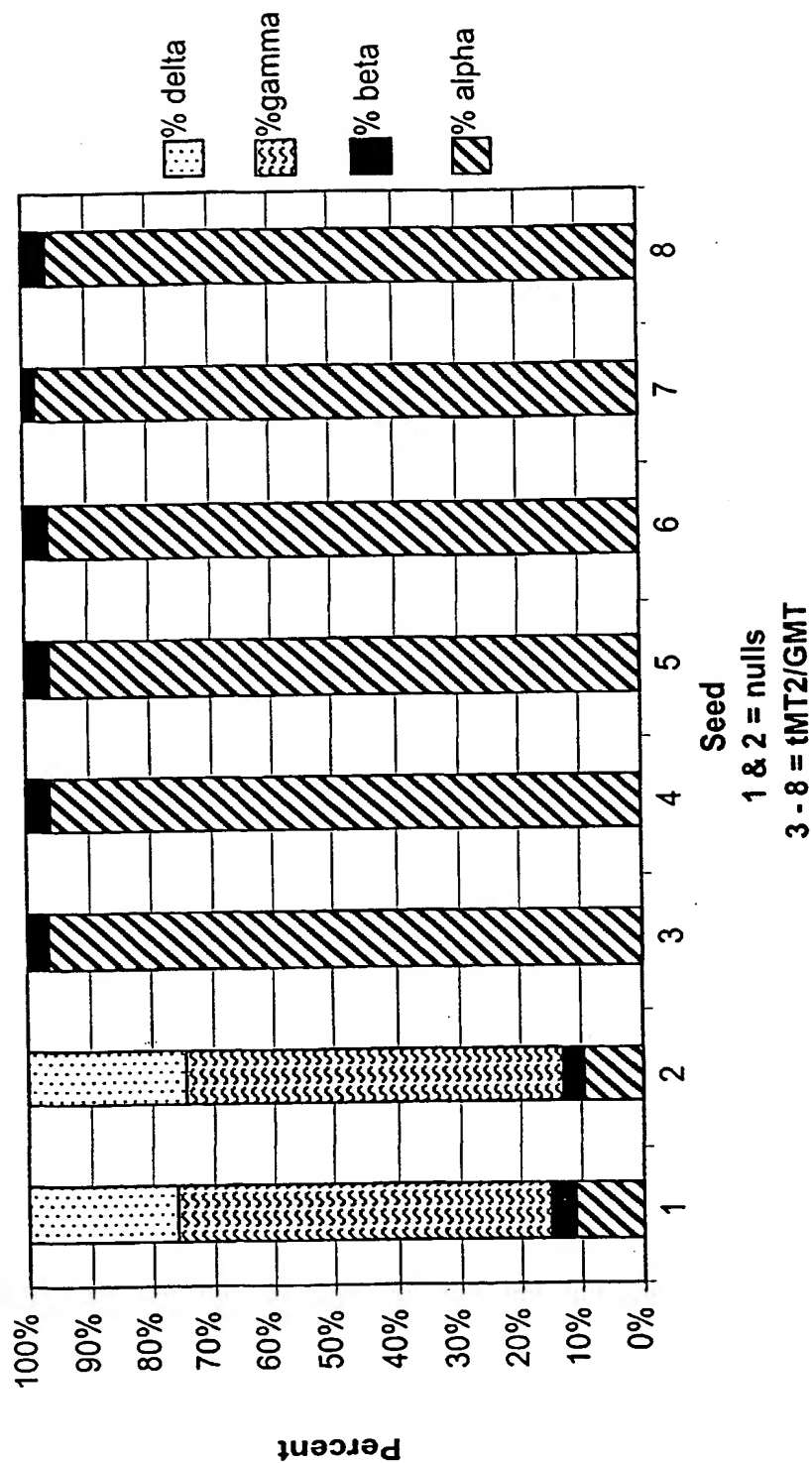
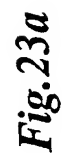
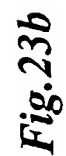
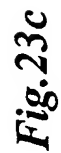
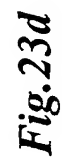


Fig. 22





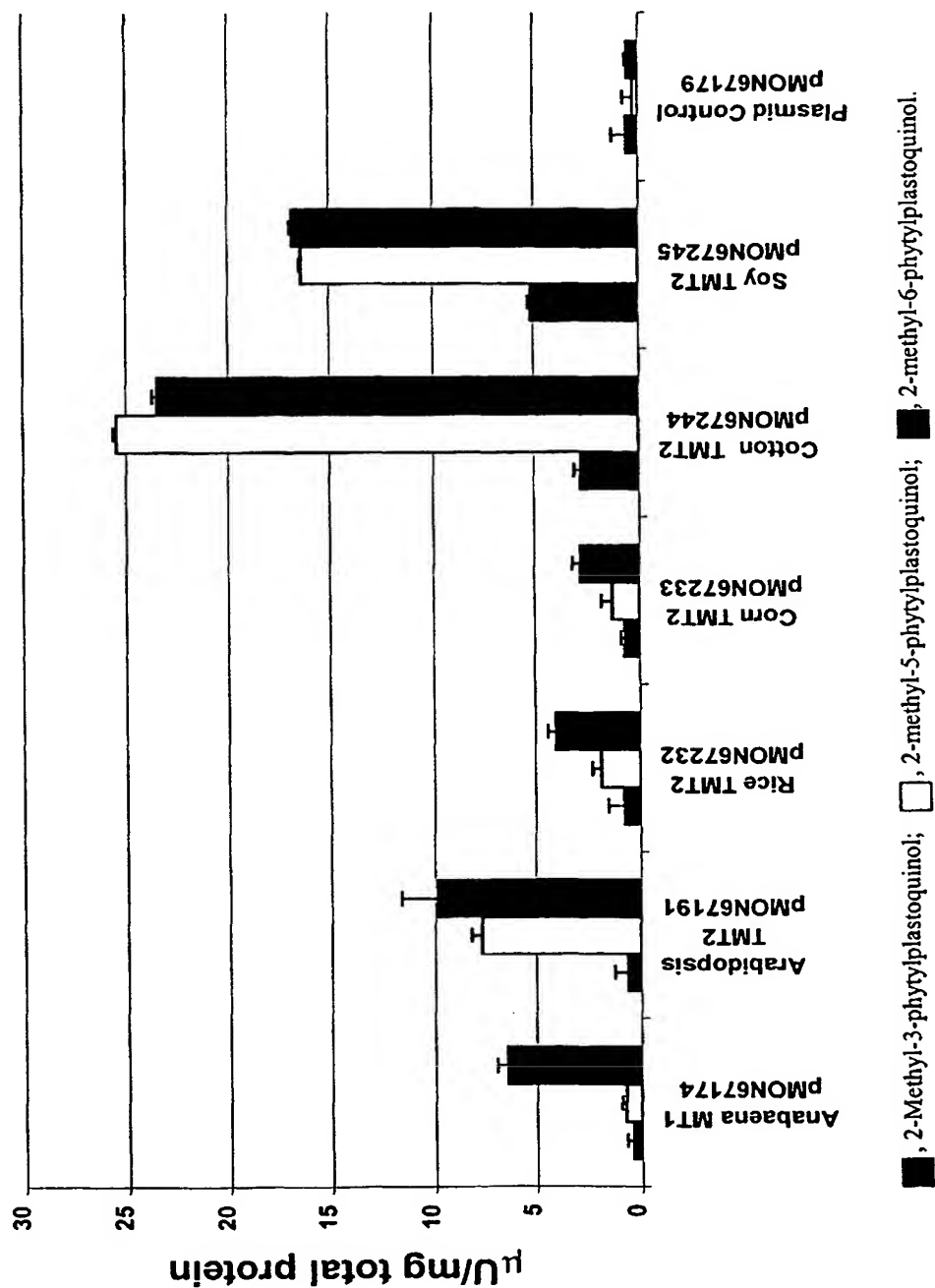


Fig. 24

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Arg Cys Ser Ser Ser Ser Val Ser Ser Ser Arg Pro Ser Ala Gln Pro
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Arg Phe Ile Gln His Lys Lys Glu Ala Tyr Trp Phe Tyr Arg Phe Leu
 65 70 75 80

Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr Glu Asp
 85 90 95

Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro Asp Met
 100 105 110

Arg Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile
 115 120 125

Val Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln Ser Pro
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His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu Cys Lys
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Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp Tyr Ala
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Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly Lys Ala
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Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg Phe Phe
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Ser Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile Glu Trp
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245 250 255

Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly Cys Ser
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Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln Leu Gly
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Pro Lys Glu Glu Asp Val Glu Lys Pro Val Asn Asn Pro Phe Ser Phe
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Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe Val Leu
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Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln Leu Gly
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Pro Lys Glu Lys Asp Val Glu Lys Pro Val Asn Asn Pro Phe Ser Phe
 290 295 300

Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe Val Leu
 305 310 315 320

Ile Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys Asp Gln
 325 330 335

Pro Ile

<210> 18

<211> 338

<212> PRT

<213> Arabidopsis thaliana

<400> 18

Met Ala Ser Leu Met Leu Asn Gly Ala Ile Thr Phe Pro Lys Gly Leu
 1 5 10 15

Gly Ser Pro Gly Ser Asn Leu His Ala Arg Ser Ile Pro Arg Pro Thr
 20 25 30

Leu Leu Ser Val Thr Arg Thr Ser Thr Pro Arg Leu Ser Val Ala Thr
 35 40 45

Arg Cys Ser Ser Ser Ser Val Ser Ser Ser Arg Pro Ser Ala Gln Pro
 50 55 60

Arg Phe Ile Gln His Lys Lys Lys Ala Tyr Trp Phe Tyr Arg Phe Leu
 65 70 75 80

Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr Glu Asp
 85 90 95

Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro Asp Met
 100 105 110

Arg Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile
 115 120 125

Val Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln Ser Pro
 130 135 140

His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu Cys Lys
 145 150 155 160

Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp Tyr Ala
 165 170 175

Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln
 180 185 190

Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly Lys Ala
 195 200 205

Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg Phe Phe
 210 215 220

Ser Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile Glu Trp
 225 230 235 240

Phe Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile Gly Pro
 245 250 255

Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly Cys Ser
 260 265 270

Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln Leu Gly
 275 280 285

Pro Lys Glu Glu Asp Val Glu Lys Pro Val Asn Asn Pro Phe Ser Phe
 290 295 300

Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe Val Leu
 305 310 315 320

Ile Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys Asp Gln
 325 330 335

Pro Ile

<210> 19
 <211> 338
 <212> PRT
 <213> Arabidopsis thaliana

<400> 19

Met Ala Ser Leu Met Leu Asn Gly Ala Ile Thr Phe Ser Lys Gly Leu
 1 5 10 15

Gly Ser Pro Gly Ser Asn Leu His Ala Arg Ser Ile Pro Arg Pro Thr
 20 25 30

Leu Leu Ser Val Thr Arg Thr Ser Thr Pro Arg Leu Ser Val Ala Thr
 35 40 45

Arg Cys Ser Ser Ser Ser Val Ser Ser Ser Arg Pro Ser Ala Gln Pro
 50 55 60

Arg Phe Ile Gln His Lys Lys Glu Ala Tyr Trp Phe Tyr Arg Phe Leu
 65 70 75 80

Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr Glu Asp
 85 90 95

Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro Asp Met
 100 105 110

Arg Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile
 115 120 125

Val Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln Ser Pro
 130 135 140

His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu Cys Lys
 145 150 155 160

Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp Tyr Ala
 165 170 175

Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln
 180 185 190

Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly Lys Ala
 195 200 205

Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg Phe Phe
 210 215 220

Ser Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile Glu Trp
 225 230 235 240

Phe Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile Gly Pro
 245 250 255

Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly Cys Ser
 260 265 270

Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln Leu Gly
 275 280 285

Pro Lys Glu Glu Asp Val Glu Lys Pro Val Asn Asn Pro Phe Ser Phe
 290 295 300

Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe Val Leu
 305 310 315 320

Ile Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys Asp Gln
 325 330 335

Pro Ile

<210> 20
 <211> 338
 <212> PRT
 <213> Arabidopsis thaliana

<400> 20

Met Ala Ser Leu Met Leu Asn Gly Ala Ile Thr Phe Pro Lys Gly Leu
 1 5 10 15

Gly Ser Pro Gly Ser Asn Leu His Ala Arg Ser Ile Pro Arg Pro Thr
 20 25 30

Leu Leu Ser Val Thr Arg Thr Ser Thr Pro Arg Leu Ser Val Ala Thr
 35 40 45

Arg Cys Ser Ser Ser Ser Val Ser Ser Ser Arg Pro Ser Ala Gln Pro
 50 55 60

Arg Phe Ile Gln His Lys Lys Glu Ala Tyr Trp Phe Tyr Arg Phe Leu
 65 70 75 80

Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr Glu Asp
 85 90 95

Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro Asp Met

100 105 110
 Arg Val Val Asn Val Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile
 115 120 125
 Val Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln Ser Pro
 130 135 140
 His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu Cys Lys
 145 150 155 160
 Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp Tyr Ala
 165 170 175
 Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln
 180 185 190
 Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly Lys Ala
 195 200 205
 Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg Phe Phe
 210 215 220
 Ser Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile Glu Trp
 225 230 235 240
 Phe Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile Gly Pro
 245 250 255
 Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly Cys Ser
 260 265 270
 Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln Leu Gly
 275 280 285
 Pro Lys Glu Glu Asp Val Glu Lys Pro Val Asn Asn Pro Phe Ser Phe
 290 295 300
 Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe Val Leu
 305 310 315 320
 Ile Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys Asp Gln
 325 330 335
 Pro Ile

<210> 21
 <211> 338
 <212> PRT
 <213> Arabidopsis thaliana

<400> 21

Met Ala Ser Leu Met Leu Asn Gly Ala Ile Thr Phe Pro Lys Gly Leu
 1 5 10 15

Gly Ser Pro Gly Ser Asn Leu His Ala Arg Ser Ile Pro Arg Pro Thr
 20 25 30

Leu Leu Ser Val Thr Arg Thr Ser Thr Pro Arg Leu Ser Val Ala Thr
 35 40 45

Arg Cys Ser Ser Ser Ser Val Ser Ser Ser Arg Pro Ser Ala Gln Pro
 50 55 60

Arg Phe Ile Gln His Lys Lys Glu Ala Tyr Trp Phe Tyr Arg Phe Leu
 65 70 75 80

Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Ile Glu Asp
 85 90 95

Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro Asp Met
 100 105 110

Arg Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile
 115 120 125

Val Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln Ser Pro
 130 135 140

His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu Cys Lys
 145 150 155 160

Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp Tyr Ala
 165 170 175

Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln
 180 185 190

Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly Lys Ala
 195 200 205

Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg Phe Phe
 210 215 220

Ser Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile Glu Trp
 225 230 235 240

Phe Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile Gly Pro
 245 250 255

Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly Cys Ser
 260 265 270

Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln Leu Gly
 275 280 285

Pro Lys Glu Glu Asp Val Glu Lys Pro Val Asn Asn Pro Phe Ser Phe
 290 295 300

Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe Val Leu
 305 310 315 320

Ile Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys Asp Gln
 325 330 335

Pro Ile

<210> 22
 <211> 352
 <212> PRT
 <213> Arabidopsis thaliana

<400> 22

Met Ala Met Ala Ser Thr Tyr Ala Pro Gly Gly Gly Ala Arg Ala Leu
 1 5 10 15

Ala Gln Gly Arg Cys Arg Val Arg Gly Pro Ala Gly Leu Gly Phe Leu
 20 25 30

Gly Pro Ser Lys Ala Ala Gly Leu Pro Arg Pro Leu Ala Leu Ala Leu
 35 40 45

Ala Arg Arg Met Ser Ser Pro Val Ala Val Gly Ala Arg Leu Arg Cys
 50 55 60

Ala Ala Ser Ser Ser Pro Ala Ala Ala Arg Pro Ala Thr Ala Pro Arg
 65 70 75 80

Phe Ile Gln His Lys Lys Glu Ala Phe Trp Phe Tyr Arg Phe Leu Ser
 85 90 95

Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr Glu Asp Met
 100 105 110

Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Phe Ser Arg His Leu Thr
 115 120 125

Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile Val
 130 135 140

Lys His Val Asn Pro Glu Asn Val Thr Leu Leu Asp Gln Ser Pro His
 145 150 155 160

Gln Leu Asp Lys Ala Arg Gln Lys Glu Ala Leu Lys Gly Val Thr Ile
 165 170 175

Met Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp Ser Phe Asp
 180 185 190

Arg Tyr Ile Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln Arg
 195 200 205

Gly Ile Lys Glu Ala Tyr Arg Val Leu Arg Phe Gly Gly Leu Ala Cys
 210 215 220

Val Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg Phe Phe Ala
 225 230 235 240

Asp Met Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile Glu Trp Phe
 245 250 255

Lys Lys Ala Gly Phe Arg Asp Val Lys Leu Lys Arg Ile Gly Pro Lys
 260 265 270

Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly Cys Ser Val
 275 280 285

Thr Gly Val Lys Arg Glu Arg Gly Asp Ser Pro Leu Glu Leu Gly Pro
 290 295 300

Lys Ala Glu Asp Val Ser Lys Pro Val Asn Pro Ile Thr Phe Leu Phe
 305 310 315 320

Arg Phe Leu Val Gly Thr Ile Cys Ala Ala Tyr Tyr Val Leu Val Pro
 325 330 335

Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys Gly Met Pro Ile
 340 345 350

<210> 23
 <211> 341
 <212> PRT
 <213> Arabidopsis thaliana

<400> 23

Met Ala Ser Ser Met Leu Asn Gly Ala Glu Thr Phe Thr Leu Ile Arg
 1 5 10 15

Gly Val Thr Pro Lys Ser Ile Gly Phe Leu Gly Ser Gly Leu His Gly
 20 25 30

Lys Gln Phe Ser Ser Ala Gly Leu Ile Tyr Ser Pro Lys Met Ser Arg
 35 40 45

Val Gly Thr Thr Ile Ala Pro Arg Cys Ser Leu Ser Ala Ser Arg Pro
 50 55 60

Ala Ser Gln Pro Arg Phe Ile Gln His Lys Lys Glu Ala Phe Trp Phe
 65 70 75 80

Tyr Arg Phe Leu Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His
 85 90 95

Trp Thr Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Asn
 100 105 110

Asp Arg Asp Met Val Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr
 115 120 125

Thr Leu Gly Ile Val Gln His Val Asp Ala Lys Asn Val Thr Ile Leu
 130 135 140

Asp Gln Ser Pro His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu
 145 150 155 160

Lys Glu Cys Asn Ile Ile Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro
 165 170 175

Thr Asp Tyr Ala Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp

180 185 190
 Pro Asp Pro Gln Arg Gly Ile Lys Glu Ala Tyr Arg Val Leu Lys Gln
 195 200 205
 Gly Gly Lys Ala Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu
 210 215 220
 Ser Arg Phe Phe Ala Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu
 225 230 235 240
 Tyr Ile Glu Trp Phe Glu Lys Ala Gly Phe Lys Asp Val Gln Leu Lys
 245 250 255
 Arg Ile Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile
 260 265 270
 Met Gly Cys Ser Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro
 275 280 285
 Leu Gln Leu Gly Pro Lys Ala Glu Asp Val Ser Lys Pro Val Asn Pro
 290 295 300
 Phe Val Phe Leu Leu Arg Phe Met Leu Gly Ala Thr Ala Ala Ala Tyr
 305 310 315 320
 Tyr Val Leu Val Pro Ile Tyr Met Trp Leu Lys Asp Gln Ile Val Pro
 325 330 335
 Glu Gly Gln Pro Ile
 340

<210> 24
 <211> 344
 <212> PRT
 <213> Arabidopsis thaliana

<400> 24

Met Ala Ser Ser Met Leu Ser Gly Ala Glu Ser Leu Ser Met Leu Arg
 1 5 10 15
 Ile His His Gln Pro Lys Leu Thr Phe Ser Ser Pro Ser Leu His Ser
 20 25 30
 Lys Pro Thr Asn Leu Lys Met Asp Leu Ile Pro Phe Ala Thr Lys His
 35 40 45

Gln Lys Thr Lys Lys Ala Ser Ile Phe Thr Cys Ser Ala Ser Ser Ser
 50 55 60

Ser Arg Pro Ala Ser Gln Pro Arg Phe Ile Gln His Lys Gln Glu Ala
 65 70 75 80

Phe Trp Phe Tyr Arg Phe Leu Ser Ile Val Tyr Asp His Val Ile Asn
 85 90 95

Pro Gly His Trp Thr Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala
 100 105 110

Glu Leu Tyr Asp Ser Arg Met Lys Val Val Asp Val Gly Gly Gly Thr
 115 120 125

Gly Phe Thr Thr Leu Gly Ile Ile Lys His Ile Asp Pro Lys Asn Val
 130 135 140

Thr Ile Leu Asp Gln Ser Pro His Gln Leu Glu Lys Ala Arg Gln Lys
 145 150 155 160

Glu Ala Leu Lys Glu Cys Thr Ile Val Glu Gly Asp Ala Glu Asp Leu
 165 170 175

Pro Phe Pro Thr Asp Thr Phe Asp Arg Tyr Val Ser Ala Gly Ser Ile
 180 185 190

Glu Tyr Trp Pro Asp Pro Gln Arg Gly Ile Lys Glu Ala Tyr Arg Val
 195 200 205

Leu Lys Leu Gly Gly Val Ala Cys Leu Ile Gly Pro Val His Pro Thr
 210 215 220

Phe Trp Leu Ser Arg Phe Phe Ala Asp Met Trp Met Leu Phe Pro Thr
 225 230 235 240

Glu Glu Glu Tyr Ile Glu Trp Phe Lys Lys Ala Gly Phe Lys Asp Val
 245 250 255

Lys Leu Lys Arg Ile Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His
 260 265 270

Gly Leu Ile Met Gly Cys Ser Val Thr Gly Val Lys Arg Leu Ser Gly
 275 280 285

Asp Ser Pro Leu Gln Leu Gly Pro Lys Ala Glu Asp Val Lys Lys Pro
 290 295 300

Ile Asn Pro Phe Ser Phe Leu Leu Arg Phe Ile Leu Gly Thr Ile Ala
 305 310 315 320

Ala Thr Tyr Tyr Val Leu Val Pro Ile Tyr Met Trp Ile Lys Asp Gln
 325 330 335

Ile Val Pro Lys Gly Gln Pro Ile
 340

<210> 25
 <211> 342
 <212> PRT
 <213> Arabidopsis thaliana

<400> 25

Met Gly Ser Val Met Leu Ser Gly Thr Glu Lys Leu Thr Leu Arg Thr
 1 5 10 15

Leu Thr Gly Asn Gly Leu Gly Phe Thr Gly Ser Asp Leu His Gly Lys
 20 25 30

Asn Phe Pro Arg Val Ser Phe Ala Ala Thr Thr Ser Ala Lys Val Pro
 35 40 45

Asn Phe Arg Ser Ile Val Val Pro Lys Cys Ser Val Ser Ala Ser Arg
 50 55 60

Pro Ser Ser Gln Pro Arg Phe Ile Gln His Lys Lys Glu Ala Phe Trp
 65 70 75 80

Phe Tyr Arg Phe Leu Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly
 85 90 95

His Trp Thr Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu
 100 105 110

Asn Asp Arg Asn Met Ile Val Val Asp Val Gly Gly Gly Thr Gly Phe
 115 120 125

Thr Thr Leu Gly Ile Val Lys His Val Asp Ala Lys Asn Val Thr Ile
 130 135 140

Leu Asp Gln Ser Pro His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro
 145 150 155 160

Leu Lys Glu Cys Lys Ile Ile Glu Gly Asp Ala Glu Asp Leu Pro Phe
 165 170 175

Arg Thr Asp Tyr Ala Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr
 180 185 190

Trp Pro Asp Pro Gln Arg Gly Ile Lys Glu Ala Tyr Arg Val Leu Lys
 195 200 205

Leu Gly Gly Lys Ala Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp
 210 215 220

Leu Ser Arg Phe Phe Ala Asp Val Trp Met Leu Phe Pro Lys Glu Glu
 225 230 235 240

Glu Tyr Ile Glu Trp Phe Gln Lys Ala Gly Phe Lys Asp Val Gln Leu
 245 250 255

Lys Arg Ile Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu
 260 265 270

Ile Met Gly Cys Ser Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser
 275 280 285

Pro Leu Gln Leu Gly Pro Lys Glu Glu Asp Val Glu Lys Pro Val Asn
 290 295 300

Pro Phe Val Phe Ala Leu Arg Phe Val Leu Gly Ala Leu Ala Ala Thr
 305 310 315 320

Trp Phe Val Leu Val Pro Ile Tyr Met Trp Leu Lys Asp Gln Val Val
 325 330 335

Pro Lys Gly Gln Pro Ile
 340

<210> 26
 <211> 348
 <212> PRT
 <213> Arabidopsis thaliana

<400> 26

Met Ala Met Ala Ser Ser Ala Tyr Ala Pro Ala Gly Gly Val Gly Thr
 1 5 10 15

His Ser Ala Pro Gly Arg Ile Arg Pro Pro Arg Gly Leu Gly Phe Ser
20 25 30

Thr Thr Thr Thr Lys Ser Arg Pro Leu Val Leu Thr Arg Arg Gly Gly
35 40 45

Gly Gly Gly Asn Ile Ser Val Ala Arg Leu Arg Cys Ala Ala Ser Ser
50 55 60

Ser Ser Ala Ala Ala Arg Pro Met Ser Gln Pro Arg Phe Ile Gln His
65 70 75 80

Lys Lys Glu Ala Phe Trp Phe Tyr Arg Phe Leu Ser Ile Val Tyr Asp
85 90 95

His Val Ile Asn Pro Gly His Trp Thr Glu Asp Met Arg Asp Asp Ala
100 105 110

Leu Glu Pro Ala Asp Leu Tyr Ser Arg Lys Leu Arg Val Val Asp Val
115 120 125

Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile Val Lys Arg Val Asp
130 135 140

Pro Glu Asn Val Thr Leu Leu Asp Gln Ser Pro His Gln Leu Glu Lys
145 150 155 160

Ala Arg Glu Lys Glu Ala Leu Lys Gly Val Thr Ile Met Glu Gly Asp
165 170 175

Ala Glu Asp Leu Pro Phe Pro Thr Asp Thr Phe Asp Arg Tyr Val Ser
180 185 190

Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln Arg Gly Ile Lys Glu
195 200 205

Ala Tyr Arg Val Leu Arg Leu Gly Gly Val Ala Cys Met Ile Gly Pro
210 215 220

Val His Pro Thr Phe Trp Leu Ser Arg Phe Phe Ala Asp Met Trp Met
225 230 235 240

Leu Phe Pro Lys Glu Glu Glu Tyr Ile Glu Trp Phe Lys Lys Ala Gly
245 250 255

Phe Lys Asp Val Lys Leu Lys Arg Ile Gly Pro Lys Trp Tyr Arg Gly

260 265 270
 Val Arg Arg His Gly Leu Ile Met Gly Cys Ser Val Thr Gly Val Lys
 275 280 285
 Arg Glu His Gly Asp Ser Pro Leu Gln Leu Gly Pro Lys Val Glu Asp
 290 295 300
 Val Ser Lys Pro Val Asn Pro Ile Thr Phe Leu Phe Arg Phe Leu Met
 305 310 315 320
 Gly Thr Ile Cys Ala Ala Tyr Tyr Val Leu Val Pro Ile Tyr Met Trp
 325 330 335
 Ile Lys Asp Gln Ile Val Pro Lys Gly Met Pro Ile
 340 345
 <210> 27
 <211> 337
 <212> PRT
 <213> Arabidopsis thaliana
 <400> 27
 Met Ala Ser Leu Met Leu Asn Gly Ala Ile Thr Phe Pro Lys Gly Leu
 1 5 10 15
 Gly Phe Pro Ala Ser Asn Leu His Ala Arg Pro Ser Pro Pro Leu Ser
 20 25 30
 Leu Val Ser Asn Thr Ala Thr Arg Arg Leu Ser Val Ala Thr Arg Cys
 35 40 45
 Ser Ser Ser Ser Ser Val Ser Ala Ser Arg Pro Ser Ala Gln Pro Arg
 50 55 60
 Phe Ile Gln His Lys Lys Glu Ala Tyr Trp Phe Tyr Arg Phe Leu Ser
 65 70 75 80
 Ile Val Tyr Asp His Ile Ile Asn Pro Gly His Trp Thr Glu Asp Met
 85 90 95
 Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro Asp Met Arg
 100 105 110
 Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile Val
 115 120 125

Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln Ser Pro His
 130 135 140

Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu Cys Lys Ile
 145 150 155 160

Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp Tyr Ala Asp
 165 170 175

Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln Arg
 180 185 190

Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly Lys Ala Cys
 195 200 205

Leu Ile Gly Pro Val His Pro Thr Phe Trp Leu Ser Arg Phe Phe Ala
 210 215 220

Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile Glu Trp Phe
 225 230 235 240

Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile Gly Pro Lys
 245 250 255

Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly Cys Ser Val
 260 265 270

Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln Leu Gly Pro
 275 280 285

Lys Glu Glu Asp Val Glu Lys Pro Val Asn Asn Pro Phe Ser Phe Leu
 290 295 300

Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe Val Leu Ile
 305 310 315 320

Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys Asp Gln Pro
 325 330 335

Ile

<210> 28
 <211> 292
 <212> PRT
 <213> Arabidopsis thaliana

<400> 28

Ala Thr Arg Cys Ser Ser Ser Ser Val Ser Ser Ser Arg Pro Ser Ala
1 5 10 15

Gln Pro Arg Phe Ile Gln His Lys Lys Glu Ala Tyr Trp Phe Tyr Arg
20 25 30

Phe Leu Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr
35 40 45

Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro
50 55 60

Asp Met Arg Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu
65 70 75 80

Gly Ile Val Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln
85 90 95

Ser Pro His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu
100 105 110

Cys Lys Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp
115 120 125

Tyr Ala Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp
130 135 140

Pro Gln Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly
145 150 155 160

Lys Ala Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg
165 170 175

Phe Phe Ser Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile
180 185 190

Glu Trp Phe Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile
195 200 205

Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly
210 215 220

Cys Ser Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln
225 230 235 240

Pro Gln Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly
145 150 155 160

Lys Ala Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg
165 170 175

Phe Phe Ser Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile
180 185 190

Glu Trp Phe Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile
195 200 205

Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly
210 215 220

Cys Ser Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln
225 230 235 240

Leu Gly Pro Lys Glu Lys Asp Val Glu Lys Pro Val Asn Asn Pro Phe
245 250 255

Ser Phe Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe
260 265 270

Val Leu Ile Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys
275 280 285

Asp Gln Pro Ile
290

<210> 30
<211> 292
<212> PRT
<213> Arabidopsis thaliana

<400> 30

Ala Thr Arg Cys Ser Ser Ser Ser Val Ser Ser Ser Arg Pro Ser Ala
1 5 10 15

Gln Pro Arg Phe Ile Gln His Lys Lys Lys Ala Tyr Trp Phe Tyr Arg
20 25 30

Phe Leu Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr
35 40 45

Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro
50 55 60

Asp Met Arg Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu
 65 70 75 80

Gly Ile Val Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln
 85 90 95

Ser Pro His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu
 100 105 110

Cys Lys Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp
 115 120 125

Tyr Ala Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp
 130 135 140

Pro Gln Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly
 145 150 155 160

Lys Ala Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg
 165 170 175

Phe Phe Ser Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile
 180 185 190

Glu Trp Phe Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile
 195 200 205

Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly
 210 215 220

Cys Ser Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln
 225 230 235 240

Leu Gly Pro Lys Glu Glu Asp Val Glu Lys Pro Val Asn Asn Pro Phe
 245 250 255

Ser Phe Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe
 260 265 270

Val Leu Ile Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys
 275 280 285

Asp Gln Pro Ile
 290

<210> 31
 <211> 292
 <212> PRT
 <213> Arabidopsis thaliana

<400> 31

Ala Thr Arg Cys Ser Ser Ser Ser Val Ser Ser Ser Arg Pro Ser Ala
 1 5 10 15

Gln Pro Arg Phe Ile Gln His Lys Lys Glu Ala Tyr Trp Phe Tyr Arg
 20 25 30

Phe Leu Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr
 35 40 45

Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro
 50 55 60

Asp Met Arg Val Val Asn Val Gly Gly Gly Thr Gly Phe Thr Thr Leu
 65 70 75 80

Gly Ile Val Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln
 85 90 95

Ser Pro His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu
 100 105 110

Cys Lys Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp
 115 120 125

Tyr Ala Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp
 130 135 140

Pro Gln Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly
 145 150 155 160

Lys Ala Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg
 165 170 175

Phe Phe Ser Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile
 180 185 190

Glu Trp Phe Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile
 195 200 205

Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly

210 215 220
 Cys Ser Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln
 225 230 235 240
 Leu Gly Pro Lys Glu Glu Asp Val Glu Lys Pro Val Asn Asn Pro Phe
 245 250 255
 Ser Phe Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe
 260 265 270
 Val Leu Ile Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys
 275 280 285
 Asp Gln Pro Ile
 290

 <210> 32
 <211> 292
 <212> PRT
 <213> Arabidopsis thaliana

 <400> 32
 Ala Thr Arg Cys Ser Ser Ser Ser Val Ser Ser Ser Arg Pro Ser Ala
 1 5 10 15
 Gln Pro Arg Phe Ile Gln His Lys Lys Glu Ala Tyr Trp Phe Tyr Arg
 20 25 30
 Phe Leu Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Ile
 35 40 45
 Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro
 50 55 60
 Asp Met Arg Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu
 65 70 75 80
 Gly Ile Val Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln
 85 90 95
 Ser Pro His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu
 100 105 110
 Cys Lys Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp
 115 120 125

Tyr Ala Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp
 130 135 140

Pro Gln Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly
 145 150 155 160

Lys Ala Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg
 165 170 175

Phe Phe Ser Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile
 180 185 190

Glu Trp Phe Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile
 195 - 200 205

Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly
 210 215 220

Cys Ser Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln
 225 230 235 240

Leu Gly Pro Lys Glu Glu Asp Val Glu Lys Pro Val Asn Asn Pro Phe
 245 250 255

Ser Phe Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe
 260 265 270

Val Leu Ile Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys
 275 280 285

Asp Gln Pro Ile
 290

<210> 33
 <211> 293
 <212> PRT
 <213> Arabidopsis thaliana

<400> 33

Ala Thr Arg Cys Ser Ser Ser Ser Ser Val Ser Ala Ser Arg Pro Ser
 1 5 10 15

Ala Gln Pro Arg Phe Ile Gln His Lys Lys Glu Ala Tyr Trp Phe Tyr
 20 25 30

Arg Phe Leu Ser Ile Val Tyr Asp His Ile Ile Asn Pro Gly His Trp

35	40	45																	
Thr	Glu	Asp	Met	Arg	Asp	Asp	Ala	Leu	Glu	Pro	Ala	Asp	Leu	Ser	His				
50						55					60								
Pro	Asp	Met	Arg	Val	Val	Asp	Val	Gly	Gly	Gly	Thr	Gly	Phe	Thr	Thr				
65					70					75					80				
Leu	Gly	Ile	Val	Lys	Thr	Val	Lys	Ala	Lys	Asn	Val	Thr	Ile	Leu	Asp				
				85					90					95					
Gln	Ser	Pro	His	Gln	Leu	Ala	Lys	Ala	Lys	Gln	Lys	Glu	Pro	Leu	Lys				
			100					105					110						
Glu	Cys	Lys	Ile	Val	Glu	Gly	Asp	Ala	Glu	Asp	Leu	Pro	Phe	Pro	Thr				
		115					120					125							
Asp	Tyr	Ala	Asp	Arg	Tyr	Val	Ser	Ala	Gly	Ser	Ile	Glu	Tyr	Trp	Pro				
130						135					140								
Asp	Pro	Gln	Arg	Gly	Ile	Arg	Glu	Ala	Tyr	Arg	Val	Leu	Lys	Ile	Gly				
145					150					155					160				
Gly	Lys	Ala	Cys	Leu	Ile	Gly	Pro	Val	His	Pro	Thr	Phe	Trp	Leu	Ser				
				165					170					175					
Arg	Phe	Phe	Ala	Asp	Val	Trp	Met	Leu	Phe	Pro	Lys	Glu	Glu	Glu	Tyr				
			180					185					190						
Ile	Glu	Trp	Phe	Lys	Asn	Ala	Gly	Phe	Lys	Asp	Val	Gln	Leu	Lys	Arg				
		195					200					205							
Ile	Gly	Pro	Lys	Trp	Tyr	Arg	Gly	Val	Arg	Arg	His	Gly	Leu	Ile	Met				
210						215					220								
Gly	Cys	Ser	Val	Thr	Gly	Val	Lys	Pro	Ala	Ser	Gly	Asp	Ser	Pro	Leu				
225					230					235					240				
Gln	Leu	Gly	Pro	Lys	Glu	Glu	Asp	Val	Glu	Lys	Pro	Val	Asn	Asn	Pro				
				245					250					255					
Phe	Ser	Phe	Leu	Gly	Arg	Phe	Leu	Leu	Gly	Thr	Leu	Ala	Ala	Ala	Trp				
			260					265					270						
Phe	Val	Leu	Ile	Pro	Ile	Tyr	Met	Trp	Ile	Lys	Asp	Gln	Ile	Val	Pro				
		275					280					285							

Lys Asp Gln Pro Ile
290

<210> 34
<211> 292
<212> PRT
<213> Arabidopsis thaliana

<400> 34

Arg Leu Arg Cys Ala Ala Ser Ser Ser Ser Ala Ala Ala Arg Pro Met
1 5 10 15

Ser Gln Pro Arg Phe Ile Gln His Lys Lys Glu Ala Phe Trp Phe Tyr
20 25 30

Arg Phe Leu Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp
35 40 45

Thr Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Tyr Ser
50 55 60

Arg Lys Leu Arg Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr
65 70 75 80

Leu Gly Ile Val Lys Arg Val Asp Pro Glu Asn Val Thr Leu Leu Asp
85 90 95

Gln Ser Pro His Gln Leu Glu Lys Ala Arg Glu Lys Glu Ala Leu Lys
100 105 110

Gly Val Thr Ile Met Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr
115 120 125

Asp Thr Phe Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro
130 135 140

Asp Pro Gln Arg Gly Ile Lys Glu Ala Tyr Arg Val Leu Arg Leu Gly
145 150 155 160

Gly Val Ala Cys Met Ile Gly Pro Val His Pro Thr Phe Trp Leu Ser
165 170 175

Arg Phe Phe Ala Asp Met Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr
180 185 190

Ile Glu Trp Phe Lys Lys Ala Gly Phe Lys Asp Val Lys Leu Lys Arg
 195 200 205

Ile Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met
 210 215 220

Gly Cys Ser Val Thr Gly Val Lys Arg Glu His Gly Asp Ser Pro Leu
 225 230 235 240

Gln Leu Gly Pro Lys Val Glu Asp Val Ser Lys Pro Val Asn Pro Ile
 245 250 255

Thr Phe Leu Phe Arg Phe Leu Met Gly Thr Ile Cys Ala Ala Tyr Tyr
 260 265 270

Val Leu Val Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys
 275 280 285

Gly Met Pro Ile
 290

<210> 35
 <211> 292
 <212> PRT
 <213> Arabidopsis thaliana

<400> 35

Arg Leu Arg Cys Ala Ala Ser Ser Ser Pro Ala Ala Ala Arg Pro Ala
 1 5 10 15

Thr Ala Pro Arg Phe Ile Gln His Lys Lys Glu Ala Phe Trp Phe Tyr
 20 25 30

Arg Phe Leu Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp
 35 40 45

Thr Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Phe Ser
 50 55 60

Arg His Leu Thr Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr
 65 70 75 80

Leu Gly Ile Val Lys His Val Asn Pro Glu Asn Val Thr Leu Leu Asp
 85 90 95

Gln Ser Pro His Gln Leu Asp Lys Ala Arg Gln Lys Glu Ala Leu Lys
 100 105 110

Gly Val Thr Ile Met Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr
 115 120 125

Asp Ser Phe Asp Arg Tyr Ile Ser Ala Gly Ser Ile Glu Tyr Trp Pro
 130 135 140

Asp Pro Gln Arg Gly Ile Lys Glu Ala Tyr Arg Val Leu Arg Phe Gly
 145 150 155 160

Gly Leu Ala Cys Val Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser
 165 170 175

Arg Phe Phe-Ala Asp Met Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr
 180 185 190

Ile Glu Trp Phe Lys Lys Ala Gly Phe Arg Asp Val Lys Leu Lys Arg
 195 200 205

Ile Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met
 210 215 220

Gly Cys Ser Val Thr Gly Val Lys Arg Glu Arg Gly Asp Ser Pro Leu
 225 230 235 240

Glu Leu Gly Pro Lys Ala Glu Asp Val Ser Lys Pro Val Asn Pro Ile
 245 250 255

Thr Phe Leu Phe Arg Phe Leu Val Gly Thr Ile Cys Ala Ala Tyr Tyr
 260 265 270

Val Leu Val Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys
 275 280 285

Gly Met Pro Ile
 290

<210> 36
 <211> 288
 <212> PRT
 <213> Arabidopsis thaliana

<400> 36

Val Pro Lys Cys Ser Val Ser Ala Ser Arg Pro Ser Ser Gln Pro Arg
 1 5 10 15

Phe Ile Gln His Lys Lys Glu Ala Phe Trp Phe Tyr Arg Phe Leu Ser
 20 25 30
 Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr Glu Asp Met
 35 40 45
 Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Asn Asp Arg Asn Met Ile
 50 55 60
 Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile Val
 65 70 75 80
 Lys His Val Asp Ala Lys Asn Val Thr Ile Leu Asp Gln Ser Pro His
 85 90 95
 Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu Cys Lys Ile
 100 105 110
 Ile Glu Gly Asp Ala Glu Asp Leu Pro Phe Arg Thr Asp Tyr Ala Asp
 115 120 125
 Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln Arg
 130 135 140
 Gly Ile Lys Glu Ala Tyr Arg Val Leu Lys Leu Gly Gly Lys Ala Cys
 145 150 155 160
 Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg Phe Phe Ala
 165 170 175
 Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile Glu Trp Phe
 180 185 190
 Gln Lys Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile Gly Pro Lys
 195 200 205
 Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly Cys Ser Val
 210 215 220
 Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln Leu Gly Pro
 225 230 235 240
 Lys Glu Glu Asp Val Glu Lys Pro Val Asn Pro Phe Val Phe Ala Leu
 245 250 255
 Arg Phe Val Leu Gly Ala Leu Ala Ala Thr Trp Phe Val Leu Val Pro

260

265

270

Ile Tyr Met Trp Leu Lys Asp Gln Val Val Pro Lys Gly Gln Pro Ile
 275 280 285

<210> 37
 <211> 289
 <212> PRT
 <213> Arabidopsis thaliana
 <400> 37

Ile Phe Thr Cys Ser Ala Ser Ser Ser Ser Arg Pro Ala Ser Gln Pro
 1 5 10 15

Arg Phe Ile Gln His Lys Gln Glu Ala Phe Trp Phe Tyr Arg Phe Leu
 20 25 30

Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr Glu Asp
 35 40 45

Met Arg Asp Asp Ala Leu Glu Pro Ala Glu Leu Tyr Asp Ser Arg Met
 50 55 60

Lys Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile
 65 70 75 80

Ile Lys His Ile Asp Pro Lys Asn Val Thr Ile Leu Asp Gln Ser Pro
 85 90 95

His Gln Leu Glu Lys Ala Arg Gln Lys Glu Ala Leu Lys Glu Cys Thr
 100 105 110

Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp Thr Phe
 115 120 125

Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln
 130 135 140

Arg Gly Ile Lys Glu Ala Tyr Arg Val Leu Lys Leu Gly Gly Val Ala
 145 150 155 160

Cys Leu Ile Gly Pro Val His Pro Thr Phe Trp Leu Ser Arg Phe Phe
 165 170 175

Ala Asp Met Trp Met Leu Phe Pro Thr Glu Glu Glu Tyr Ile Glu Trp
 180 185 190

Phe Lys Lys Ala Gly Phe Lys Asp Val Lys Leu Lys Arg Ile Gly Pro
 195 200 205

Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly Cys Ser
 210 215 220

Val Thr Gly Val Lys Arg Leu Ser Gly Asp Ser Pro Leu Gln Leu Gly
 225 230 235 240

Pro Lys Ala Glu Asp Val Lys Lys Pro Ile Asn Pro Phe Ser Phe Leu
 245 250 255

Leu Arg Phe Ile Leu Gly Thr Ile Ala Ala Thr Tyr Tyr Val Leu Val
 260 265 270

Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys Gly Gln Pro
 275 280 285

Ile

<210> 38
 <211> 288
 <212> PRT
 <213> Arabidopsis thaliana

<400> 38

Ala Pro Arg Cys Ser Leu Ser Ala Ser Arg Pro Ala Ser Gln Pro Arg
 1 5 10 15

Phe Ile Gln His Lys Lys Glu Ala Phe Trp Phe Tyr Arg Phe Leu Ser
 20 25 30

Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr Glu Asp Met
 35 40 45

Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Asn Asp Arg Asp Met Val
 50 55 60

Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile Val
 65 70 75 80

Gln His Val Asp Ala Lys Asn Val Thr Ile Leu Asp Gln Ser Pro His
 85 90 95

Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu Cys Asn Ile

100 105 110
 Ile Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp Tyr Ala Asp
 115 120 125
 Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln Arg
 130 135 140
 Gly Ile Lys Glu Ala Tyr Arg Val Leu Lys Gln Gly Gly Lys Ala Cys
 145 150 155 160
 Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg Phe Phe Ala
 165 170 175
 Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile Glu Trp Phe
 180 185 190
 Glu Lys Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile Gly Pro Lys
 195 200 205
 Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly Cys Ser Val
 210 215 220
 Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln Leu Gly Pro
 225 230 235 240
 Lys Ala Glu Asp Val Ser Lys Pro Val Asn Pro Phe Val Phe Leu Leu
 245 250 255
 Arg Phe Met Leu Gly Ala Thr Ala Ala Ala Tyr Tyr Val Leu Val Pro
 260 265 270
 Ile Tyr Met Trp Leu Lys Asp Gln Ile Val Pro Glu Gly Gln Pro Ile
 275 280 285

<210> 39
 <211> 1047
 <212> DNA
 <213> Arabidopsis thaliana

<400> 39
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 tcttttcggct caaagtcacg gcttctcttt cgggtctccat cctcctcctc ctcagtctct 120
 atgacgacaa cgcgtggaaa cgtggctgtg gcggctgctg ctacatccac tgaggcgcta 180
 agaaaaggaa tagcggagtt ctacaatgaa acttcggggt tgtgggaaga gatttgggga 240

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gatcatatgc atcatggctt ttatgaccct gattcttctg ttcaactttc tgattctggt 300
cacaaggaag ctcatgccg tatgattgaa gagtctctcc gtttcgccgg tgttactgat 360
gaagaggagg agaaaaagat aaagaaagta gtggatgttg ggtgtgggat tggaggaagc 420
tcaagatatc ttgcctctaa atttggagct gaatgcattg gcattactct cagccctggt 480
caggccaaga gagccaatga tctcgcggt gctcaatcac tctctcataa ggcttccttc 540
caagttgcgg atgcgttgga tcagccattc gaagatggaa aattcgatct agtgtggtcg 600
atggagagtg gtgagcatat gcctgacaag gccaaagttg taaaagagtt ggtacgtgtg 660
gcggtccag gaggtaggat aataatagt acatggtgcc atagaaatct atctgcgggg 720
gaggaagctt tgcagccgtg ggagcaaac atcttgaca aaatctgtaa gacgttctat 780
ctcccggtt ggtgctccac cgatgattat gtcaacttgc ttcaatccca ttctctccag 840
gatattaagt gtgcggattg gtcagagAAC gtatctcctt tctggcctgc ggttatacgg 900
actgcattaa catggaagg ccttgtgtct ctgcttcgta gtggtatgaa aagtattaaa 960
ggagcattga caatgccatt gatgattgaa ggttacaaga aaggtgtcat taagtttggt 1020
atcatcactt gccagaagcc actctaa 1047

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<210> 40

<211> 1047

<212> DNA

<213> Arabidopsis thaliana

<400> 40

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tctttcggtt caaagtcac gcttctcttt cggctctccat cctcctctc ctcatctct 120
atgacgacaa cgcgtggaaa cgtggctgtg gcggctgctg ctacatccac tgaggcgcta 180
agaaaaggaa tagcggagtt ctacaatgaa acttcgggtt tgtgggaaga gatttgggga 240
gatcatatgc atcatggctt ttatgaccct gattcttctg ttcaactttc tgattctggt 300
cacaaggaag ctcatgccg tatgattgaa gagtctctcc gttttgccgg tgttactgat 360
gaagaggagg agaaaaagat aaagaaagta gtggatgttg ggtgtgggat tggaggaagc 420
tcaagatatc ttgcctctaa atttggagct gaatgcattg gcattactct cagccctggt 480
caggccaaga gagccaatga tctcgcggt gctcaatcac tcgctcataa ggcttccttc 540
caagttgcgg atgcgttgga tcagccattc gaagatggaa aattcgatct agtgtggtcg 600
atggagagtg gtgagcatat gcctgacaag gccaaagttg taaaagagtt ggtacgtgtg 660
gcggctccag gaggtaggat aataatagt acatggtgcc atagaaatct atctgcgggg 720
gaggaagctt tgcagccgtg ggagcaaac atcttgaca aaatctgtaa gacgttctat 780

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ctccccggctt ggtgctccac cgatgattat gtcaacttgc ttcaatccca ttctctccag      840
gatattaagt gtgcggtattg gtcagagaac gtagctcctt tctggcctgc gggtatacgg      900
actgcattaa catggaaggg ccttgtgtct ctgcttcgta gtggtatgaa aagtattaaa      960
ggagcattga caatgccatt gatgattgaa ggttacaaga aaggtgtcat taagtttggt    1020
atcatcactt gccagaagcc actctaa                                           1047

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<210> 41
 <211> 1095
 <212> DNA
 <213> *Arabidopsis thaliana*

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<400> 41
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cgccgcccgc gccgcagcag ccggaggacg aaactcgccg tgcgcgcgat ggcaccgacg    180
ttgtcctcgt cgtcgacggc ggcggcagct cccccggggc tgaaggaggg catcgcgggg    240
ctctacgacg agtcgtccgg cgtgtgggag agcatctggg gcgagcacat gcaccacggc    300
ttctacgacg ccggcgaggc cgcctccatg tccgaccacc gccgcgcca gatccgcatg    360
atcgaggaat ccctcgctt cgccgccgtc cccggtgcag atgatgcgga gaagaaaccc    420
aaaagtgtag ttgatgttg ctgtggcatt ggtggtagct caagatactt ggcgaacaaa    480
tacggagcgc aatgctacg catcacgtt agtccggtgc aggctgaaag aggaaatgcc    540
ctcgcggcag agcaagggtt atcagacaag gtgcgtattc aagttggtga tgcattggag    600
cagccttttc ctgatggga gtttgatctt gtctgggtcca tggagagtgg cgagcacatg    660
ccagacaaac ggcagtttgt aagcgagctg gcacgcgtcg cagctcctgg ggcgagaata    720
atcattgtga cctggtgcc taggaacctc gagccatccg aagagtcctt gaaacctgat    780
gagctgaatc tcctgaaaag gatatgcgat gcatattatc tcccagactg gtgctctcct    840
tctgattatg tcaaaattgc cgagtcactg tctcttgagg atataaggac agctgattgg    900
tcagagaacg tcgccccatt ctggcctgcg gttataaaat cagcattgac atggaaaggt    960
ttaacttctc tgctaagaag tgggtggaag acgataagag gtgcaatggt gatgcctctg   1020
atgatcgaag gatacaagaa agggctcatc aaattcacca tcatcacctg tcgcaagccc   1080
gaaacaacgc agtag                                                         1095

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<210> 42
 <211> 1059
 <212> DNA
 <213> *Arabidopsis thaliana*

<400> 42
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 gccgtcgta gcctgcgtcc gatggcctcg tcgacggctc agggcccccgc gacggcgccg 180
 ccgggtctga aggagggcat cgcggggctg tacgacgagt cgtcggggct gtgggagaac 240
 atctggggcg accacatgca ccacggcttc tacgactcga gcgaggccgc ctccatggcc 300
 gatcaccgcc gcgccagat ccgcatgac gagggaggcg tcgccttcgc cgggtgtccca 360
 gcctcagatg atccagagaa gacacaaaa acaatagtcg atgtcggatg tggcattggt 420
 ggtagctcaa ggtacttggc gaagaaatac ggagcgagc gactgggat cacgttgagc 480
 cctgttcaag ccgagagagg aatgctctc gctgcagcgc aggggttgct ggatcaggtt 540
 actctgcaag ttgctgatgc tctggagcaa ccgtttcctg acgggcagtt cgatctggtg 600
 tgggccatgg agagtggcga gcacatgccg gacaagagaa agtttgtag tgagctagca 660
 cgctggcggt ctctggagg gacaataatc atcgtgacat ggtgccatag gaacctggat 720
 ccatccgaaa cctcgctaaa gcccgatgaa ctgagcctcc tgaggaggat atgcgacgcg 780
 tactacctcc cggactggtg ctacacctca gactatgtga acattgccaa gtcactgtct 840
 ctcgaggata tcaagacagc tgactggctg gagaacgtgg ccccgttttg gcccgccgtg 900
 ataaaatcag cgctaacatg gaagggttc acctctctgc tgacgaccgg atggaagacg 960
 atcagaggcg cgatggtgat gccgctaag atccagggt acaagaagg gctcatcaaa 1020
 ttcaccatca tcacctgtcg caagcctgga gccgcgtag 1059

<210> 43
 <211> 1038
 <212> DNA
 <213> Arabidopsis thaliana

<400> 43
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 cgttcctgt cgcgcatgt gaaggcgcg gcgtcgctct tgtccaccac caccttgag 180
 gaagggatag cggagtttta cgatgagtcg tcggggattt gggaagacat atgggggtgac 240
 catatgcacc atggatatta cgagccgggt tccgatattt cgggttcaga tcacgtgccc 300
 gctcagattc gaatggtcga agaatcgctc cgttttgctg gaatatcaga ggaccagca 360
 aacaggccca agagaatagt tgatgttggg tgtgggatag gaggcagttc taggtatcta 420
 gcaaggaaat atggggcaaa atgccaggc attactttga gccctgttca agctggaaga 480
 gccaatgctc ttgctaagtc tcaaggacta gcagaacagg tttgttttga agttgcagat 540

gccttgaacc aaccattccc tgatgaccaa tttgatcttg tttggtctat ggaaagcgga 600
gaacacatgc ctgacaaaacc caagtttggt aaagagctgg tgcgagtggc agctccagga 660
ggcacaataa tagtagtgac atggtgccat agggatcttg gtccatctga agagtctttg 720
cagccatggg agcaaaagct tttaaacaga atatgtgatg cttactatct accagagtgg 780
tgttctactt ctgattatgt caaattatct cagtccctat ctctccagga tataaaggca 840
ggagactgga ctgagaatgt agcacccttt tggccagcag tgatacggtc agcattgaca 900
tggaagggtc tcacatcgct gctacgaagt ggattaaaa caataaaagg tgcaactggtg 960
atgccattga tgatcgaagg tttccagaaa ggggtgataa agtttgccat cattgcttgc 1020
cggaagccag ctgagtag 1038

<210> 44
<211> 1131
<212> DNA
<213> Arabidopsis thaliana

<400> 44
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aagagcaaca agaaccacgg cttgggtcgg tccggtttctg gttggagaag gatgtttggg 180
acatgggcta ctgccgacaa gactcagagt accgatacgt ctaatgaagg cgtgggttagt 240
tacgatactc aggtcttgca gaagggtata gcggagttct atgacgagtc gtcgggtata 300
tgggaggata tatggggaga tcacatgcat catggctact atgatgggtc cactcctgtc 360
tcccctccag accatcgctc tgcgcagatc cgaatgattg acgaggctct ccgctttgcc 420
tcggttcctt caggagaaga agatgagtcc aagtctaaga ttccaaagag gatagtggat 480
gtcgggtgtg ggataggggg aagctccaga tacctggcta gaaaatatgg cgccgagtgt 540
cggggcatca ctctcagtc tgtccaggct gagaggggca attcacttgc acggtctcaa 600
gggtctttctg acaagggtct ctttcaagtc gccgatgctt tggcacagcc atttcccgat 660
ggacagtttg atttgggtct gtccatggag agcgggggaa acatgcccga caagagcaag 720
tttgtcaatg agctagtaag agtagcagct ccgggtggca cgataataat tgtcacatgg 780
tgccatagag atctcaggga agacgaagat gcgctgcagc ctgaggagaa agagatattg 840
gacaagatat gcaaccctt ttatcttccc gcctgggtgt ctgctgccga ctatgttaag 900
ttgtctcagt cacttgatgt cgaggacatt aaatctgcgg actggactcc atatgttgcc 960
ccattttggc cagctgtgct gaagtccgct ttcactataa agggcttcgt gtctctattg 1020
aggagcggaa tgaagaccat aaaggagca tttgcaatgc cgctgatgat cgaaggatac 1080

aagaaaggtg tcatcaagtt ttccatcatc acatgccgta agcccgaata g 1131

<210> 45

<211> 2045

<212> DNA

<213> Arabidopsis thaliana

<400> 45

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1053

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 <213> Arabidopsis thaliana

<400> 57
 tgtaaaacga cggccagtag ctatgcggat tgatggte 38

<210> 58
 <211> 38
 <212> DNA
 <213> Arabidopsis thaliana

<400> 58
 caggaaacag ctatgacctc ctctgggaa ctctagca 38

<210> 59
<211> 38
<212> DNA
<213> Arabidopsis thaliana

<400> 59
tgtaaaacga cggccagttg ctgacttgcg agtttttg 38

<210> 60
<211> 38
<212> DNA
<213> Arabidopsis thaliana

<400> 60
caggaaacag ctatgacccc tgtcaacaac cccttctc 38

<210> 61
<211> 39
<212> DNA
<213> Arabidopsis thaliana

<400> 61
tgtaaaacga cggccagtcc acaagagggg tttacaatg 39

<210> 62
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<212> DNA
<213> Arabidopsis thaliana

<400> 62
caggaaacag ctatgaccac ccaaccttct ggctctct 38

<210> 63
<211> 38
<212> DNA
<213> Arabidopsis thaliana

<400> 63
tgtaaaacga cggccagtgg tctttgggaa cgatctga 38

<210> 64
<211> 38
<212> DNA
<213> Arabidopsis thaliana

<400> 64
caggaaacag ctatgaccag ggaagcgtac agggttct 38

<210> 65
<211> 38
<212> DNA
<213> Arabidopsis thaliana

<400> 65

tgtaaaacga cggccagtc tcttgagctg aacgtcct

38

<210> 66
<211> 38
<212> DNA
<213> Arabidopsis thaliana

<400> 66
caggaaacag ctatgaccgg cggaactggt ttcactac

38

<210> 67
<211> 38
<212> DNA
<213> Arabidopsis thaliana

<400> 67
tgtaaaacga cggccagttg tcagcataat cggttgga

38

<210> 68
<211> 38
<212> DNA
<213> Arabidopsis thaliana

<400> 68
caggaaacag ctatgacctc cccaaagggt taggttcc

38

<210> 69
<211> 38
<212> DNA
<213> Arabidopsis thaliana

<400> 69
tgtaaaacga cggccagtaa gcctccttct tgtgctga

38

<210> 70
<211> 38
<212> DNA
<213> Arabidopsis thaliana

<400> 70
caggaaacag ctatgaccgg acttttcct tccatttg

38

<210> 71
<211> 38
<212> DNA
<213> Arabidopsis thaliana

<400> 71
tgtaaaacga cggccagttg gaggttcggg taactgag

38

<210> 72
<211> 38
<212> DNA
<213> Arabidopsis thaliana

<400> 72
caggaaacag ctatgaccga tcctctcgct agcaggctc 38

<210> 73
<211> 38
<212> DNA
<213> Arabidopsis thaliana

<400> 73
tgtaaaacga cggccagtgg aaccagggga acctaaac 38

<210> 74
<211> 38
<212> DNA
<213> Arabidopsis thaliana

<400> 74
caggaaacag ctatgaccgc cgtgagaaac agactcct 38

<210> 75
<211> 38
<212> DNA
<213> Arabidopsis thaliana

<400> 75
tgtaaaacga cggccagtca aatggaaggg aaaagtgc 38

<210> 76
<211> 38
<212> DNA
<213> Arabidopsis thaliana

<400> 76
caggaaacag ctatgaccga tccaaagaga acccagca 38

<210> 77
<211> 74
<212> DNA
<213> Arabidopsis thaliana

<400> 77
gggacaagtt tgtacaaaaa agcaggctta gaaggagata gaaccatggc gacaagatgc 60
agcagcagca gcag 74

<210> 78
<211> 62
<212> DNA
<213> Arabidopsis thaliana

<400> 78
ggggaccact ttgtacaaga aagctgggtc ctgcagggtca gatgggttgg tctttgggaa 60
cg 62

<210> 79
 <211> 72
 <212> DNA
 <213> Arabidopsis thaliana

<400> 79
 gggacaagtt tgtacaaaaa agcaggctta gaaggagata gaaccatgcg gctgaggtgc 60
 gcggcgtcgt cg 72

<210> 80
 <211> 61
 <212> DNA
 <213> Arabidopsis thaliana

<400> 80
 ggggaccact ttgtacaaga aagctgggtc ctgcagggtta gatcggcatg cctttgggca 60
 c 61

<210> 81
 <211> 72
 <212> DNA
 <213> Arabidopsis thaliana

<400> 81
 gggacaagtt tgtacaaaaa agcaggctta gaaggagata gaaccatgag gctgcgatgc 60
 gcggcgtcgt cg 72

<210> 82
 <211> 62
 <212> DNA
 <213> Arabidopsis thaliana

<400> 82
 ggggaccact ttgtacaaga aagctgggtc ctgcagggtca gattggcatg ccttttggca 60
 cg 62

<210> 83
 <211> 71
 <212> DNA
 <213> Arabidopsis thaliana

<400> 83
 gggacaagtt tgtacaaaaa agcaggctta gaaggagata gaaccatggt acccaagtgt 60
 agtgtctcgg c 71

<210> 84
 <211> 61
 <212> DNA
 <213> Arabidopsis thaliana

<400> 84
 ggggaccact ttgtacaaga aagctgggtc ctgcagggtta gattggctga cttttgggaa 60
 c 61

<210> 85
 <211> 70
 <212> DNA
 <213> Arabidopsis thaliana

<400> 85
 gggacaagtt tgtacaaaaa agcagggtta gaaggagata gaaccatgat ctttacatgc 60
 agcgcgtcct 70

<210> 86
 <211> 61
 <212> DNA
 <213> Arabidopsis thaliana

<400> 86
 ggggaccact ttgtacaaga aagctgggtc ctgcagggtca tatgggctgg cttttcggtta 60
 c 61

<210> 87
 <211> 72
 <212> DNA
 <213> Arabidopsis thaliana

<400> 87
 gggacaagtt tgtacaaaaa agcagggtta gaaggagata gaaccatggc cccgagggtgc 60
 agcttatcag cg 72

<210> 88
 <211> 61
 <212> DNA
 <213> Arabidopsis thaliana

<400> 88
 ggggaccact ttgtacaaga aagctgggtc ctgcagggtta gattggttga ccctctggta 60
 c 61

<210> 89
 <211> 65
 <212> DNA
 <213> Arabidopsis thaliana

<400> 89
 ggggacaagt ttgtacaaaa aagcagggtg cggccgctga acaatggcct ctttgatgct 60
 caacg 65

<210> 90
 <211> 62
 <212> DNA
 <213> Arabidopsis thaliana

<400> 90
 ggggaccact ttgtacaaga aagctggggtc ctgcaggtca gatggggttg tctttgggaa 60
 cg 62

<210> 91
 <211> 348
 <212> PRT
 <213> Arabidopsis thaliana

<400> 91

Met Lys Ala Thr Leu Ala Ala Pro Ser Ser Leu Thr Ser Leu Pro Tyr
 1 5 10 15

Arg Thr Asn Ser Ser Phe Gly Ser Lys Ser Ser Leu Leu Phe Arg Ser
 20 25 30

Pro Ser Ser Ser Ser Ser Val Ser Met Thr Thr Thr Arg Gly Asn Val
 35 40 45

Ala Val Ala Ala Ala Ala Thr Ser Thr Glu Ala Leu Arg Lys Gly Ile
 50 55 60

Ala Glu Phe Tyr Asn Glu Thr Ser Gly Leu Trp Glu Glu Ile Trp Gly
 65 70 75 80

Asp His Met His His Gly Phe Tyr Asp Pro Asp Ser Ser Val Gln Leu
 85 90 95

Ser Asp Ser Gly His Lys Glu Ala Gln Ile Arg Met Ile Glu Glu Ser
 100 105 110

Leu Arg Phe Ala Gly Val Thr Asp Glu Glu Glu Glu Lys Lys Ile Lys
 115 120 125

Lys Val Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu
 130 135 140

Ala Ser Lys Phe Gly Ala Glu Cys Ile Gly Ile Thr Leu Ser Pro Val
 145 150 155 160

Gln Ala Lys Arg Ala Asn Asp Leu Ala Ala Ala Gln Ser Leu Ser His
 165 170 175

Lys Ala Ser Phe Gln Val Ala Asp Ala Leu Asp Gln Pro Phe Glu Asp
 180 185 190

Gly Lys Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro
 195 200 205

Asp Lys Ala Lys Phe Val Lys Glu Leu Val Arg Val Ala Ala Pro Gly
 210 215 220

Gly Arg Ile Ile Ile Val Thr Trp Cys His Arg Asn Leu Ser Ala Gly
 225 230 235 240

Glu Glu Ala Leu Gln Pro Trp Glu Gln Asn Ile Leu Asp Lys Ile Cys
 245 250 255

Lys Thr Phe Tyr Leu Pro Ala Trp Cys Ser Thr Asp Asp Tyr Val Asn
 260 265 270

Leu Leu Gln Ser His Ser Leu Gln Asp Ile Lys Cys Ala Asp Trp Ser
 275 280 285

Glu Asn Val Ala Pro Phe Trp Pro Ala Val Ile Arg Thr Ala Leu Thr
 290 295 300

Trp Lys Gly Leu Val Ser Leu Leu Arg Ser Gly Met Lys Ser Ile Lys
 305 310 315 320

Gly Ala Leu Thr Met Pro Leu Met Ile Glu Gly Tyr Lys Lys Gly Val
 325 330 335

Ile Lys Phe Gly Ile Ile Thr Cys Gln Lys Pro Leu
 340 345

<210> 92

<211> 348

<212> PRT

<213> Arabidopsis thaliana

<400> 92

Met Lys Ala Thr Leu Ala Ala Pro Ser Ser Leu Thr Ser Leu Pro Tyr
 1 5 10 15

Arg Thr Asn Ser Ser Phe Gly Ser Lys Ser Ser Leu Leu Phe Arg Ser
 20 25 30

Pro Ser Ser Ser Ser Ser Val Ser Met Thr Thr Thr Arg Gly Asn Val
 35 40 45
 Ala Val Ala Ala Ala Ala Thr Ser Thr Glu Ala Leu Arg Lys Gly Ile
 50 55 60
 Ala Glu Phe Tyr Asn Glu Thr Ser Gly Leu Trp Glu Glu Ile Trp Gly
 65 70 75 80
 Asp His Met His His Gly Phe Tyr Asp Pro Asp Ser Ser Val Gln Leu
 85 90 95
 Ser Asp Ser Gly His Lys Glu Ala Gln Ile Arg Met Ile Glu Glu Ser
 100 105 110
 Leu Arg Phe Ala Gly Val Thr Asp Glu Glu Glu Glu Lys Lys Ile Lys
 115 120 125
 Lys Val Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu
 130 135 140
 Ala Ser Lys Phe Gly Ala Glu Cys Ile Gly Ile Thr Leu Ser Pro Val
 145 150 155 160
 Gln Ala Lys Arg Ala Asn Asp Leu Ala Ala Ala Gln Ser Leu Ala His
 165 170 175
 Lys Ala Ser Phe Gln Val Ala Asp Ala Leu Asp Gln Pro Phe Glu Asp
 180 185 190
 Gly Lys Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro
 195 200 205
 Asp Lys Ala Lys Phe Val Lys Glu Leu Val Arg Val Ala Ala Pro Gly
 210 215 220
 Gly Arg Ile Ile Ile Val Thr Trp Cys His Arg Asn Leu Ser Ala Gly
 225 230 235 240
 Glu Glu Ala Leu Gln Pro Trp Glu Gln Asn Ile Leu Asp Lys Ile Cys
 245 250 255
 Lys Thr Phe Tyr Leu Pro Ala Trp Cys Ser Thr Asp Asp Tyr Val Asn
 260 265 270
 Leu Leu Gln Ser His Ser Leu Gln Asp Ile Lys Cys Ala Asp Trp Ser

275 280 285
 Glu Asn Val Ala Pro Phe Trp Pro Ala Val Ile Arg Thr Ala Leu Thr
 290 295 300
 Trp Lys Gly Leu Val Ser Leu Leu Arg Ser Gly Met Lys Ser Ile Lys
 305 310 315 320
 Gly Ala Leu Thr Met Pro Leu Met Ile Glu Gly Tyr Lys Lys Gly Val
 325 330 335
 Ile Lys Phe Gly Ile Ile Thr Cys Gln Lys Pro Leu
 340 345
 <210> 93
 <211> 364
 <212> PRT
 <213> Oryza sativa
 <400> 93
 Met Ala His Ala Ala Ala Ala Thr Gly Ala Leu Ala Pro Leu His Pro
 1 5 10 15
 Leu Leu Arg Cys Thr Ser Arg His Leu Cys Ala Ser Ala Ser Pro Arg
 20 25 30
 Ala Gly Leu Cys Leu His His His Arg Arg Arg Arg Arg Ser Ser Arg
 35 40 45
 Arg Thr Lys Leu Ala Val Arg Ala Met Ala Pro Thr Leu Ser Ser Ser
 50 55 60
 Ser Thr Ala Ala Ala Ala Pro Pro Gly Leu Lys Glu Gly Ile Ala Gly
 65 70 75 80
 Leu Tyr Asp Glu Ser Ser Gly Val Trp Glu Ser Ile Trp Gly Glu His
 85 90 95
 Met His His Gly Phe Tyr Asp Ala Gly Glu Ala Ala Ser Met Ser Asp
 100 105 110
 His Arg Arg Ala Gln Ile Arg Met Ile Glu Glu Ser Leu Ala Phe Ala
 115 120 125
 Ala Val Pro Gly Ala Asp Asp Ala Glu Lys Lys Pro Lys Ser Val Val
 130 135 140

Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu Ala Asn Lys
 145 150 155 160

Tyr Gly Ala Gln Cys Tyr Gly Ile Thr Leu Ser Pro Val Gln Ala Glu
 165 170 175

Arg Gly Asn Ala Leu Ala Ala Glu Gln Gly Leu Ser Asp Lys Val Arg
 180 185 190

Ile Gln Val Gly Asp Ala Leu Glu Gln Pro Phe Pro Asp Gly Gln Phe
 195 200 205

Asp Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro Asp Lys Arg
 210 215 220

Gln Phe Val Ser Glu Leu Ala Arg Val Ala Ala Pro Gly Ala Arg Ile
 225 230 235 240

Ile Ile Val Thr Trp Cys His Arg Asn Leu Glu Pro Ser Glu Glu Ser
 245 250 255

Leu Lys Pro Asp Glu Leu Asn Leu Leu Lys Arg Ile Cys Asp Ala Tyr
 260 265 270

Tyr Leu Pro Asp Trp Cys Ser Pro Ser Asp Tyr Val Lys Ile Ala Glu
 275 280 285

Ser Leu Ser Leu Glu Asp Ile Arg Thr Ala Asp Trp Ser Glu Asn Val
 290 295 300

Ala Pro Phe Trp Pro Ala Val Ile Lys Ser Ala Leu Thr Trp Lys Gly
 305 310 315 320

Leu Thr Ser Leu Leu Arg Ser Gly Trp Lys Thr Ile Arg Gly Ala Met
 325 330 335

Val Met Pro Leu Met Ile Glu Gly Tyr Lys Lys Gly Leu Ile Lys Phe
 340 345 350

Thr Ile Ile Thr Cys Arg Lys Pro Glu Thr Thr Gln
 355 360

<210> 94
 <211> 352
 <212> PRT
 <213> Zea mays

<400> 94

Met Ala His Ala Ala Leu Leu His Cys Ser Gln Ser Ser Arg Ser Leu
 1 5 10 15

Ala Ala Cys Arg Arg Gly Ser His Tyr Arg Ala Pro Ser His Val Pro
 20 25 30

Arg His Ser Arg Arg Leu Arg Arg Ala Val Val Ser Leu Arg Pro Met
 35 40 45

Ala Ser Ser Thr Ala Gln Ala Pro Ala Thr Ala Pro Pro Gly Leu Lys
 50 55 60

Glu Gly Ile Ala Gly Leu Tyr Asp Glu Ser Ser Gly Leu Trp Glu Asn
 65 70 75 80

Ile Trp Gly Asp His Met His His Gly Phe Tyr Asp Ser Ser Glu Ala
 85 90 95

Ala Ser Met Ala Asp His Arg Arg Ala Gln Ile Arg Met Ile Glu Glu
 100 105 110

Ala Leu Ala Phe Ala Gly Val Pro Ala Ser Asp Asp Pro Glu Lys Thr
 115 120 125

Pro Lys Thr Ile Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg
 130 135 140

Tyr Leu Ala Lys Lys Tyr Gly Ala Gln Cys Thr Gly Ile Thr Leu Ser
 145 150 155 160

Pro Val Gln Ala Glu Arg Gly Asn Ala Leu Ala Ala Ala Gln Gly Leu
 165 170 175

Ser Asp Gln Val Thr Leu Gln Val Ala Asp Ala Leu Glu Gln Pro Phe
 180 185 190

Pro Asp Gly Gln Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His
 195 200 205

Met Pro Asp Lys Arg Lys Phe Val Ser Glu Leu Ala Arg Val Ala Ala
 210 215 220

Pro Gly Gly Thr Ile Ile Ile Val Thr Trp Cys His Arg Asn Leu Asp
 225 230 235 240

Pro Ser Glu Thr Ser Leu Lys Pro Asp Glu Leu Ser Leu Leu Arg Arg
 245 250 255

Ile Cys Asp Ala Tyr Tyr Leu Pro Asp Trp Cys Ser Pro Ser Asp Tyr
 260 265 270

Val Asn Ile Ala Lys Ser Leu Ser Leu Glu Asp Ile Lys Thr Ala Asp
 275 280 285

Trp Ser Glu Asn Val Ala Pro Phe Trp Pro Ala Val Ile Lys Ser Ala
 290 295 300

Leu Thr Trp Lys Gly Phe Thr Ser Leu Leu Thr Thr Gly Trp Lys Thr
 305 310 315 320

Ile Arg Gly Ala Met Val Met Pro Leu Met Ile Gln Gly Tyr Lys Lys
 325 330 335

Gly Leu Ile Lys Phe Thr Ile Ile Thr Cys Arg Lys Pro Gly Ala Ala
 340 345 350

<210> 95

<211> 345

<212> PRT

<213> Gossypium hirsutum

<400> 95

Met Ala Ala Ala Leu Gln Leu Gln Thr His Pro Cys Phe His Gly Thr
 1 5 10 15

Cys Gln Leu Ser Pro Pro Pro Arg Pro Ser Val Ser Phe Pro Ser Ser
 20 25 30

Ser Arg Ser Phe Pro Ser Ser Arg Arg Ser Leu Ser Ala His Val Lys
 35 40 45

Ala Ala Ala Ser Ser Leu Ser Thr Thr Thr Leu Gln Glu Gly Ile Ala
 50 55 60

Glu Phe Tyr Asp Glu Ser Ser Gly Ile Trp Glu Asp Ile Trp Gly Asp
 65 70 75 80

His Met His His Gly Tyr Tyr Glu Pro Gly Ser Asp Ile Ser Gly Ser
 85 90 95

Asp His Arg Ala Ala Gln Ile Arg Met Val Glu Glu Ser Leu Arg Phe
 100 105 110

Ala Gly Ile Ser Glu Asp Pro Ala Asn Arg Pro Lys Arg Ile Val Asp
 115 120 125

Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu Ala Arg Lys Tyr
 130 135 140

Gly Ala Lys Cys Gln Gly Ile Thr Leu Ser Pro Val Gln Ala Gly Arg
 145 150 155 160

Ala Asn Ala Leu Ala Asn Ala Gln Gly Leu Ala Glu Gln Val Cys Phe
 165 170 175

Glu Val Ala Asp Ala Leu Asn Gln Pro Phe Pro Asp Asp Gln Phe Asp
 180 185 190

Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro Asp Lys Pro Lys
 195 200 205

Phe Val Lys Glu Leu Val Arg Val Ala Ala Pro Gly Gly Thr Ile Ile
 210 215 220

Val Val Thr Trp Cys His Arg Asp Leu Gly Pro Ser Glu Glu Ser Leu
 225 230 235 240

Gln Pro Trp Glu Gln Lys Leu Leu Asn Arg Ile Cys Asp Ala Tyr Tyr
 245 250 255

Leu Pro Glu Trp Cys Ser Thr Ser Asp Tyr Val Lys Leu Phe Gln Ser
 260 265 270

Leu Ser Leu Gln Asp Ile Lys Ala Gly Asp Trp Thr Glu Asn Val Ala
 275 280 285

Pro Phe Trp Pro Ala Val Ile Arg Ser Ala Leu Thr Trp Lys Gly Phe
 290 295 300

Thr Ser Leu Leu Arg Ser Gly Leu Lys Thr Ile Lys Gly Ala Leu Val
 305 310 315 320

Met Pro Leu Met Ile Glu Gly Phe Gln Lys Gly Val Ile Lys Phe Ala
 325 330 335

Ile Ile Ala Cys Arg Lys Pro Ala Glu

340

345

<210> 96
 <211> 376
 <212> PRT
 <213> cuphea pulcherrima

<400> 96

Met Pro Ile Thr Ser Ile Ser Ala Asn Gln Arg Pro Phe Phe Pro Ser
 1 5 10 15

Pro Tyr Arg Gly Ser Ser Lys Asn Met Ala Pro Pro Glu Leu Ala Gln
 20 25 30

Ser Gln Val Pro Met Gly Ser Asn Lys Ser Asn Lys Asn His Gly Leu
 35 40 45

Val Gly Ser Val Ser Gly Trp Arg Arg Met Phe Gly Thr Trp Ala Thr
 50 55 60

Ala Asp Lys Thr Gln Ser Thr Asp Thr Ser Asn Glu Gly Val Val Ser
 65 70 75 80

Tyr Asp Thr Gln Val Leu Gln Lys Gly Ile Ala Glu Phe Tyr Asp Glu
 85 90 95

Ser Ser Gly Ile Trp Glu Asp Ile Trp Gly Asp His Met His His Gly
 100 105 110

Tyr Tyr Asp Gly Ser Thr Pro Val Ser Leu Pro Asp His Arg Ser Ala
 115 120 125

Gln Ile Arg Met Ile Asp Glu Ala Leu Arg Phe Ala Ser Val Pro Ser
 130 135 140

Gly Glu Glu Asp Glu Ser Lys Ser Lys Ile Pro Lys Arg Ile Val Asp
 145 150 155 160

Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu Ala Arg Lys Tyr
 165 170 175

Gly Ala Glu Cys Arg Gly Ile Thr Leu Ser Pro Val Gln Ala Glu Arg
 180 185 190

Gly Asn Ser Leu Ala Arg Ser Gln Gly Leu Ser Asp Lys Val Ser Phe
 195 200 205

Gln Val Ala Asp Ala Leu Ala Gln Pro Phe Pro Asp Gly Gln Phe Asp
 210 215 220

Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro Asp Lys Ser Lys
 225 230 235 240

Phe Val Asn Glu Leu Val Arg Val Ala Ala Pro Gly Gly Thr Ile Ile
 245 250 255

Ile Val Thr Trp Cys His Arg Asp Leu Arg Glu Asp Glu Asp Ala Leu
 260 265 270

Gln Pro Arg Glu Lys Glu Ile Leu Asp Lys Ile Cys Asn Pro Phe Tyr
 275 280 285

Leu Pro Ala Trp Cys Ser Ala Ala Asp Tyr Val Lys Leu Leu Gln Ser
 290 295 300

Leu Asp Val Glu Asp Ile Lys Ser Ala Asp Trp Thr Pro Tyr Val Ala
 305 310 315 320

Pro Phe Trp Pro Ala Val Leu Lys Ser Ala Phe Thr Ile Lys Gly Phe
 325 330 335

Val Ser Leu Leu Arg Ser Gly Met Lys Thr Ile Lys Gly Ala Phe Ala
 340 345 350

Met Pro Leu Met Ile Glu Gly Tyr Lys Lys Gly Val Ile Lys Phe Ser
 355 360 365

Ile Ile Thr Cys Arg Lys Pro Glu
 370 375

<210> 97
 <211> 347
 <212> PRT
 <213> Brassica napus

<400> 97

Met Lys Ala Thr Leu Ala Pro Ser Ser Leu Ile Ser Leu Pro Arg His
 1 5 10 15

Lys Val Ser Ser Leu Arg Ser Pro Ser Leu Leu Leu Gln Ser Gln Arg
 20 25 30

Pro Ser Ser Ala Leu Met Thr Thr Thr Thr Ala Ser Arg Gly Ser Val

35	40	45
Ala Val Thr Ala Ala Ala Thr Ser Ser Val Glu Ala Leu Arg Glu Gly		
50	55	60
Ile Ala Glu Phe Tyr Asn Glu Thr Ser Gly Leu Trp Glu Glu Ile Trp		
65	70	75
Gly Asp His Met His His Gly Phe Tyr Asp Pro Asp Ser Ser Val Gln		
85	90	95
Leu Ser Asp Ser Gly His Arg Glu Ala Gln Ile Arg Met Ile Glu Glu		
100	105	110
Ser Leu Arg Phe Ala Gly Val Thr Glu Glu Glu Lys Lys Ile Lys Arg		
115	120	125
Val Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Ile Ala		
130	135	140
Ser Lys Phe Gly Ala Glu Cys Ile Gly Ile Thr Leu Ser Pro Val Gln		
145	150	155
Ala Lys Arg Ala Asn Asp Leu Ala Ala Ala Gln Ser Leu Ser His Lys		
165	170	175
Val Ser Phe Gln Val Ala Asp Ala Leu Glu Gln Pro Phe Glu Asp Gly		
180	185	190
Ile Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro Asp		
195	200	205
Lys Ala Lys Phe Val Lys Glu Leu Val Arg Val Ala Ala Pro Gly Gly		
210	215	220
Arg Ile Ile Ile Val Thr Trp Cys His Arg Asn Leu Ser Pro Gly Glu		
225	230	235
Glu Ala Leu Gln Pro Trp Glu Gln Asn Leu Leu Asp Arg Ile Cys Lys		
245	250	255
Thr Phe Tyr Leu Pro Ala Trp Cys Ser Thr Ser Asp Tyr Val Asp Leu		
260	265	270
Leu Gln Ser Leu Ser Leu Gln Asp Ile Lys Cys Ala Asp Trp Ser Glu		
275	280	285

Asn Val Ala Pro Phe Trp Pro Ala Val Ile Arg Thr Ala Leu Thr Trp
 290 295 300

Lys Gly Leu Val Ser Leu Leu Arg Ser Gly Met Lys Ser Ile Lys Gly
 305 310 315 320

Ala Leu Thr Met Pro Leu Met Ile Glu Gly Tyr Lys Lys Gly Val Ile
 325 330 335

Lys Phe Gly Ile Ile Thr Cys Gln Lys Pro Leu
 340 345

<210> 98

<211> 347

<212> PRT

<213> Brassica napus

<400> 98

Met Lys Ala Thr Leu Ala Pro Pro Ser Ser Leu Ile Ser Leu Pro Arg
 1 5 10 15

His Lys Val Ser Ser Leu Arg Ser Pro Ser Leu Leu Leu Gln Ser Gln
 20 25 30

Arg Arg Ser Ser Ala Leu Met Thr Thr Thr Ala Ser Arg Gly Ser Val
 35 40 45

Ala Val Thr Ala Ala Ala Thr Ser Ser Ala Glu Ala Leu Arg Glu Gly
 50 55 60

Ile Ala Glu Phe Tyr Asn Glu Thr Ser Gly Leu Trp Glu Glu Ile Trp
 65 70 75 80

Gly Asp His Met His His Gly Phe Tyr Asp Pro Asp Ser Ser Val Gln
 85 90 95

Leu Ser Asp Ser Gly His Arg Glu Ala Gln Ile Arg Met Ile Glu Glu
 100 105 110

Ser Leu Arg Phe Ala Gly Val Thr Glu Glu Glu Lys Lys Ile Lys Arg
 115 120 125

Val Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Ile Ala
 130 135 140

Ser Lys Phe Gly Ala Glu Cys Ile Gly Ile Thr Leu Ser Pro Val Gln
 145 150 155 160

Ala Lys Arg Ala Asn Asp Leu Ala Thr Ala Gln Ser Leu Ser His Lys
 165 170 175

Val Ser Phe Gln Val Ala Asp Ala Leu Asp Gln Pro Phe Glu Asp Gly
 180 185 190

Ile Ser Asp Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro Asp
 195 200 205

Lys Ala Lys Phe Val Lys Glu Leu Val Arg Val Thr Ala Pro Gly Gly
 210 215 220

Arg Ile Ile Ile Val Thr Trp Cys His Arg Asn Leu Ser Gln Gly Glu
 225 230 235 240

Glu Ser Leu Gln Pro Trp Glu Gln Asn Leu Leu Asp Arg Ile Cys Lys
 245 250 255

Thr Phe Tyr Leu Pro Ala Trp Cys Ser Thr Thr Asp Tyr Val Glu Leu
 260 265 270

Leu Gln Ser Leu Ser Leu Gln Asp Ile Lys Tyr Ala Asp Trp Ser Glu
 275 280 285

Asn Val Ala Pro Phe Trp Pro Ala Val Ile Arg Thr Ala Leu Thr Trp
 290 295 300

Lys Gly Leu Val Ser Leu Leu Arg Ser Gly Met Lys Ser Ile Lys Gly
 305 310 315 320

Ala Leu Thr Met Pro Leu Met Ile Glu Gly Tyr Lys Lys Gly Val Ile
 325 330 335

Lys Phe Gly Ile Ile Thr Cys Gln Lys Pro Leu
 340 345

<210> 99

<211> 310

<212> PRT

<213> Lycopersicon esculentum

<400> 99

Met Ala Ser Val Ala Ala Met Asn Ala Val Ser Ser Ser Ser Val Glu
 1 5 10 15

Val Gly Ile Gln Asn Gln Gln Glu Leu Lys Lys Gly Ile Ala Asp Leu
 20 25 30

Tyr Asp Glu Ser Ser Gly Ile Trp Glu Asp Ile Trp Gly Asp His Met
 35 40 45

His His Gly Tyr Tyr Glu Pro Lys Ser Ser Val Glu Leu Ser Asp His
 50 55 60

Arg Ala Ala Gln Ile Arg Met Ile Glu Gln Ala Leu Ser Phe Ala Ala
 65 70 75 80

Ile Ser Glu Asp Pro Ala Lys Lys Pro Thr Ser Ile Val Asp Val Gly
 85 90 95

Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu Ala Lys Lys Tyr Gly Ala
 100 105 110

Thr Ala Lys Gly Ile Thr Leu Ser Pro Val Gln Ala Glu Arg Ala Gln
 115 120 125

Ala Leu Ala Asp Ala Gln Gly Leu Gly Asp Lys Val Ser Phe Gln Val
 130 135 140

Ala Asp Ala Leu Asn Gln Pro Phe Pro Asp Gly Gln Phe Asp Leu Val
 145 150 155 160

Trp Ser Met Glu Ser Gly Glu His Met Pro Asn Lys Glu Lys Phe Val
 165 170 175

Gly Glu Leu Ala Arg Val Ala Ala Pro Gly Gly Thr Ile Ile Leu Val
 180 185 190

Thr Trp Cys His Arg Asp Leu Ser Pro Ser Glu Glu Ser Leu Thr Pro
 195 200 205

Glu Glu Lys Glu Leu Leu Asn Lys Ile Cys Lys Ala Phe Tyr Leu Pro
 210 215 220

Ala Trp Cys Ser Thr Ala Asp Tyr Val Lys Leu Leu Gln Ser Asn Ser
 225 230 235 240

Leu Gln Asp Ile Lys Ala Glu Asp Trp Ser Glu Asn Val Ala Pro Phe
 245 250 255

Trp Pro Ala Val Ile Lys Ser Ala Leu Thr Trp Lys Gly Phe Thr Ser
 260 265 270

Val Leu Arg Ser Gly Trp Lys Thr Ile Lys Ala Ala Leu Ala Met Pro
 275 280 285

Leu Met Ile Glu Gly Tyr Lys Lys Gly Leu Ile Lys Phe Ala Ile Ile
 290 295 300

Thr Cys Arg Lys Pro Glu
 305 310

<210> 100

<211> 302

<212> PRT

<213> GLYCINE MAX

<400> 100

Met Ser Val Glu Gln Lys Ala Ala Gly Lys Glu Glu Glu Gly Lys Leu
 1 5 10 15

Gln Lys Gly Ile Ala Glu Phe Tyr Asp Glu Ser Ser Gly Ile Trp Glu
 20 25 30

Asn Ile Trp Gly Asp His Met His His Gly Phe Tyr Asp Pro Asp Ser
 35 40 45

Thr Val Ser Val Ser Asp His Arg Ala Ala Gln Ile Arg Met Ile Gln
 50 55 60

Glu Ser Leu Arg Phe Ala Ser Leu Leu Ser Glu Asn Pro Ser Lys Trp
 65 70 75 80

Pro Lys Ser Ile Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg
 85 90 95

Tyr Leu Ala Lys Lys Phe Gly Ala Thr Ser Val Gly Ile Thr Leu Ser
 100 105 110

Pro Val Gln Ala Gln Arg Ala Asn Ala Leu Ala Ala Ala Gln Gly Leu
 115 120 125

Ala Asp Lys Val Ser Phe Gln Val Ala Asp Ala Leu Gln Gln Pro Phe
 130 135 140

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Xaa Xaa Cys Xaa Xaa Xaa Xaa Ser Xaa Xaa Xaa Xaa Arg Pro Xaa Xaa	65	70	80
Xaa Pro Arg Phe Ile Gln His Lys Xaa Glu Ala Xaa Trp Phe Tyr Arg	85	90	95
Phe Leu Ser Ile Val Tyr Asp His Xaa Ile Asn Pro Gly His Trp Thr	100	105	110
Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala Xaa Leu Xaa Xaa Xaa	115	120	125
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 325 330 335

Val Leu Xaa Pro Ile Tyr Met Trp Xaa Lys Asp Gln Xaa Val Pro Xaa
 340 345 350

Xaa Xaa Pro Ile
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(19) World Intellectual Property
Organization
International Bureau



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1 May 2003 (01.05.2003)

PCT

(10) International Publication Number
WO 2003/034812 A3

- (51) International Patent Classification⁷: **C12N 15/29**, 15/82, A01H 5/00, 5/10
- (21) International Application Number: **PCT/US2002/034079**
- (22) International Filing Date: 24 October 2002 (24.10.2002)
- (25) Filing Language: English
- (26) Publication Language: English
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60/330,563 25 October 2001 (25.10.2001) US
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- (74) Agent: **HANSON, Robert, E.**; Fullbright & Jaworski L.L.P., 600 Congress Avenue, Suite 2400, Austin, TX 78701 (US).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**
— with international search report
- (88) Date of publication of the international search report: 26 February 2004
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: AROMATIC METHYLTRANSFERASES AND USES THEREOF

(57) Abstract: The present invention relates to genes associated with the tocopherol biosynthesis pathway. More particularly, the present invention provides and includes nucleic acid molecules, proteins, and antibodies associated with genes that encode polypeptides that have methyltransferase activity. The present invention also provides methods for utilizing such agents, for example in gene isolation, gene analysis and the production of transgenic plants. Moreover, the present invention includes transgenic plants modified to express the aforementioned polypeptides. In addition, the present invention includes methods for the production of products from the tocopherol biosynthesis pathway.

WO 2003/034812 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/34079

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 15/29, 15/82; A01H 5/00, 5/10

US CL : 536/23.6; 800/278, 298, 305, 306, 312, 313, 314, 315, 316, 317, 317.1, 317.2, 317.4, 319, 320, 320.1, 320.2, 320.3, 323

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.6; 800/278, 298, 305, 306, 312, 313, 314, 315, 316, 317, 317.1, 317.2, 317.4, 319, 320, 320.1, 320.2, 320.3, 323

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00/10380 A1 (UNIVERSITY OF NEVADA) 02 March 2000 (02.03.2000), page 24 claim 2 and sequence listing SEQ ID NO:1.	1-2



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

17 June 2003 (17.06.2003)

Date of mailing of the international search report

28 JUL 2003

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Facsimile No. (703)305-3230

Authorized officer.

Felicia D. Roberts for
Cynthia Collins

Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/34079

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-3,14-20,27-32,38-42,48-49,52-53,56-57,65 and SEQ ID NO:16

Remark on Protest

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☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I-XXIII, claim(s) 1-3, 14-20, 27-32, 38-42, 48-49, 52-53, 56-57 and 65, drawn to a substantially pure nucleic acid molecule encoding a plant polypeptide having 2-methylphytylplastoquinol methyltransferase activity, a transformed plant, and a method of producing a plant. Group I is directed to a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence of SEQ ID NO:16, Group II is directed to a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence of SEQ ID NO:17, ... Group XXIII is directed to a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence of SEQ ID NO:38.

Groups XXIV-XXVIII, claim(s) 1 and 4-5, drawn to a substantially pure nucleic acid molecule encoding a plant polypeptide having 2-methylphytylplastoquinol methyltransferase activity. Group XXIV is directed to a nucleic acid molecule that is a mutant hdt2 gene, Group XXV is directed to a nucleic acid molecule that is a mutant hdt6 gene, Group XXVI is directed to a nucleic acid molecule that is a mutant hdt9 gene, Group XXVII is directed to a nucleic acid molecule that is a mutant hdt10 gene, Group XXVIII is directed to a nucleic acid molecule that is a mutant hdt16 gene.

Groups XXIX-XL claim(s) 6, 9-13, 21-26, 33-37, 43-47, 50-51, 54-55, 58-64 and 70, drawn to a substantially pure nucleic acid molecule encoding a plant polypeptide having 2-methylphytylplastoquinol methyltransferase activity, a transformed plant, a method of reducing in a plant expression of a gene, and a method of producing a plant. Group XXIX is directed to a nucleic acid molecule comprising a nucleic acid sequence of SEQ ID NO:3, Group XXX is directed to a nucleic acid molecule comprising a nucleic acid sequence of SEQ ID NO:4 ... Group XXXX is directed to a nucleic acid molecule comprising a nucleic acid sequence of SEQ ID NO:14.

Groups XLI-XLVIII, claim(s) 7-8, drawn to a substantially purified plant polypeptide having 2-methylphytylplastoquinol methyltransferase activity. Group XLI is directed to a polypeptide native to *Arabidopsis thaliana*, Columbia ecotype, Group XLII is directed to a polypeptide native to *Arabidopsis thaliana*, Landsburg ecotype, Group XLIII is directed to a polypeptide native to corn, Group XLIV is directed to a polypeptide native to soybean, Group XLV is directed to a polypeptide native to rice, Group XLVI is directed to a polypeptide native to *Allium*, Group XLVII is directed to a polypeptide native to *Brassica*, Group XLVIII is directed to a polypeptide native to *Gossypium*.

Groups XLIX-LI, claim(s) 9-13, 21-26, 33-37, 43-47, 50-51, 54-55, 58-64 and 70, drawn to a transformed plant, a method of reducing in a plant expression of a gene, and a method of producing a plant. Group XLIX is directed to a transformed plant comprising a nucleic acid sequence of SEQ ID NO:1, Group L is directed to a transformed plant comprising a nucleic acid sequence of SEQ ID NO:2, Group LI is directed to a transformed plant comprising a nucleic acid sequence SEQ ID NO:15.

Group LII, claim(s) 66-67, drawn to a transformed plant comprising a first nucleic acid molecule encoding an MT2 enzyme and a second nucleic acid molecule encoding a GMT enzyme, and a method of producing a plant.

Groups LIII-LXII, claim(s) 68, drawn to oil. Group LIII is directed to oil from a seed comprising a nucleic acid sequence of SEQ ID NO:1, Group LIV is directed to oil from a seed comprising a nucleic acid sequence of SEQ ID NO:2, ... Group LXII is directed to oil from a seed comprising a nucleic acid sequence of SEQ ID NO:15.

Groups LXIII-LXXII, claim(s) 69, drawn to animal feed. Group LXIII is directed to animal feed from a seed comprising a nucleic acid sequence of SEQ ID NO:1, Group LXIV is directed to animal feed from a seed comprising a nucleic acid sequence of SEQ ID NO:2, ... Group LXXII is directed to animal feed from a seed comprising a nucleic acid sequence of SEQ ID NO:15.

The inventions listed as Groups I-XXXXVII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking Groups I-XL, XLIX-LI and LXIII-LXXII appears to be a substantially pure nucleic acid molecule encoding a plant polypeptide having 2-methylphytylplastoquinol methyltransferase activity. However, WO 00/10380 (International

INTERNATIONAL SEARCH REPORT

PCT/US02/34079

Publication Date 2 March 2000) teach or suggest a substantially pure nucleic acid molecule encoding a plant polypeptide having 2-methylphytylplastoquinol methyltransferase activity (page 6 lines 22-32). Therefore, the technical feature linking Groups I-XL, XLIX-LI and LXIII-LXXII does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art. Accordingly, Groups Groups I-XL, XLIX-LI and LXIII-LXXII are not so linked by the same or corresponding special technical feature so as to form a single general inventive concept. Furthermore, the special technical feature of each of Groups I-XL, XLIX-LI and LXIII-LXXII is the specific polynucleotide sequence of each Group. Additionally, the special technical feature of Groups XLI-XLVIII is the specific polypeptide of each Group, and the special technical feature of Group LII is the combination of a first nucleic acid molecule encoding an MT2 enzyme and a second nucleic acid molecule encoding a GMT enzyme.

Continuation of B. FIELDS SEARCHED Item 3:

WEST/STN (agricola, biosis, biotechno, caba, caplus, medline): inventor names, 2-methylphytylplastoquinol, methyltransferase, plant, transgenic, DNA; STIC sequence search for SEQ ID NO:16.

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